

**COMPARATIVE STUDY OF ANTIHYPERGLYCAEMIC ACTIVITY
OF *URTICA DIOICA* (STINGING NETTLE), *AZADIRACHTA INDICA* (NEEM)
AND *ALOE SECUNDIFLORA* (ALOE) EXTRACTS ON RABBITS AND
STREPTOZOTOCIN INDUCED DIABETIC RATS**

NJOROGE JANE WANJIKU

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*This thesis is dedicated to my dear husband Ephantus,
daughter Samantha, sons David and Job.*

DECLARATION

This thesis is my original work and has not been presented for award of a degree in any other university.

NJOROGE JANE WANJIKUSignature..... .....

Date 13.11.2012

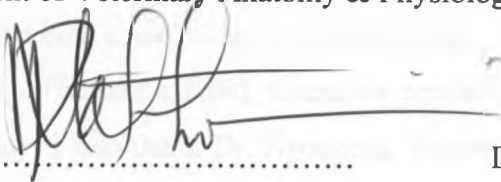
This thesis has been submitted for examination with our approval as university supervisors

DR. JACQUES KABARU

School of Biological Sciences

Signature..... .....Date .. 13th November, 2012**DR. MBAABU MATHIU**

Department of Veterinary Anatomy & Physiology

Signature..... .....

Date .. 13/11/2012

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LIST OF ABBREVIATIONS AND SYMBOLS

ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
ANOVA	Analysis of variance
cAMP	cyclic adenosine monophosphate
CMC	Carboxymethyl Cellulose
d.f	Degrees of freedom
DAG	Diacylglycerol
g	Gram
i.p.	intraperitoneal
KCl	Potassium Chloride
K ATP channels	ATP dependent Potassium channels
KRB	Krebs Ringer bicarbonate
MgCl ₂ .6H ₂ O	Magnesium Chloride
NaCl	Sodium Chloride
NaHCO ₃	Sodium bicarbonate
NaH ₂ PO ₄ .2H ₂ O	Sodium dihydrogen Phosphate
nm	Nanometre
PBS	Phosphate Buffered Saline
®	Registered trademark
NAD	Nicotine Amine Dinucleotide
PARP	Poly-(ADP-ribose) polymerase
rpm	Revolutions per minute
STZ	Streptozotocin
μl	Microlitre
μg	Microgram
™	Trademark
°C	Degrees Celsius
V / W	Volume to weight

ABSTRACT

The prevalence of type 2 diabetes is increasing in developing, as well as other countries. Searching for hypoglycaemic agents with origin from domestic herbals was considered a useful and promising way to find novel therapy of the disease. This study was designed to investigate the antihyperglycaemic potential of *Aloe secundiflora*, *Urtica dioica* and *Azadirachta indica* leaves and assess various dose-response effects in the rabbit and diabetic Wistar rats. Shadow dried and powdered *A. indica* and *U. dioica* leaf were extracted using 60% ethyl alcohol (1:3 w/v). Dried *A. secundiflora* leaf sap juice was concentrated in an open vessel. Their formulation mode was improved by constituting syrup. Diabetes was induced by a single i.p streptozotocin injection (50 mg/kg). Graded doses of the extracts and placebo were orally administered to normoglycaemic rabbits for 4 weeks. In oral glucose tolerance test male Wistar rats were administered glucose (3g/kg b.w) 210 min after oral administration of extracts (80 mg kg⁻¹). 80 mg kg⁻¹ b.w of the extracts was administered orally to diabetic rats for 6 weeks. Control animals were given glibenclamide and placebo. Blood samples were evaluated using a glucose oxidase kit, (Human, Wiesbaden Germany). At the end of 6 weeks, the rats were sacrificed by anaesthesia, whole pancreas and kidney extracted, weighed, fixed in Bouin's solution and stained by hematoxylin –eosin dye. There was noticeable reduction in blood glucose levels after chronic administration of *A. indica* and *U. dioica* extract at 10, 20, 40 and 80 mg kg⁻¹ in normoglycaemic rabbits. *A. secundiflora* did not show any effect at these doses. 100 mg kg⁻¹ *A. indica* and *U. dioica* extracts had statistically significant effect, $p < 0.05$ against the control. Animals fed on *A. secundiflora* had decreased appetite and experienced loose stool. In OGTT model the blood glucose levels of groups fed on *A. secundiflora*, *U. dioica* and *A. indica* were $p < 0.001$, $p < 0.001$ and $p < 0.05$ respectively compared to control group at 120 min. *A. secundiflora*, *U. dioica* and *A. indica* leaf extracts also produced a significant decrease in glucose level ($p < 0.05$) of diabetic-treated compared with diabetic controls. In segment jejunum the percentage fall in glucose concentration of the medium with initial values was 27.7% for *A. secundiflora*, 31.6% *A. indica* and 36.0% for *U. dioica* against 43.8% for the control during 2 hours. The results indicate that *A. secundiflora*, *A. indica* and *U. dioica* are potential antihyperglycaemics and can be used in modulating blood glucose levels.

Key words: Comparative antihyperglycaemic, *Aloe secundiflora*, *Urtica dioica*, *Azadirachta indica*, Dose-response, Stz-Wistar rats.

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Background information

Diabetes affects one hundred and thirty five million people in one year worldwide (King *et. al.*, 1999) and this figure is projected to rise to three hundred million in 2025 ((King *et. al.*, 1998). It is obvious that diabetes, a chronic non communicable disease, continues to have a tremendous impact on society in terms of the quality of life and straining health care resources. The costs incurred in managing or preventing it are enormous, both in Kenya and throughout the world. The disease causes substantial morbidity, mortality and long-term complications and remains a risk factor for cardiovascular disease. In Africa, this disease continues to impact on the poverty levels of the people.

Diabetes is a complex group of disorders that is most commonly characterized by hyperglycaemia, with the disorder affecting the metabolism of carbohydrate, fats and proteins. The disorder results from an inability to control blood glucose levels due to insufficient levels or activity of insulin. Elevated glucose levels in turn, often lead to secondary health problems that require additional medical treatment. The leading diabetic health risks include hyperglycaemia, arteriosclerosis, diabetic retinopathy (possibly leading to blindness), cataracts, nephropathy, increased risk of infections, hypertension, neuropathy, risk of amputations, impotence and diabetic ketoacidosis.

There are two primary types of diabetes, type 1 and type II diabetes (The centers for disease control and prevention, 1998). Type I diabetes generally occurs in childhood and results from the body's inability to produce insulin. The etiology of type 2 diabetes is complex involving progressive development of insulin resistance and relative deficiency in insulin secretion leading to overt hyperglycaemia (Ostenson, 2001), and increased amyloid deposition in the pancreatic islets. Other subtypes of diabetes mellitus are gestational and condition specific types.

Management of diabetes appeared a distinct possibility in 1921 with the discovery of insulin. However, this proved not to be the case as insulin's side effects were manifested after use. Insulin is expensive, way beyond the reach of most average diabetics. Oral pharmacological agents used to control diabetes have also been developed and even though they are effective in controlling hyperglycaemia, they have prominent side effects. Most of them, suffer from inadequate long- term efficacy and treatment failure, after a long time of treatment (Deforms, 1999). Thus continuous treatment modifications and monitoring are often necessary. Moreover, with increasing reliance on multiple patented pharmacological agents, the cost of treatment has also become a real concern and the ability of developing countries to afford this level of treatment is straining.

Thus, there remains a need in the art to develop methods of alleviating, preventing or treating symptoms of diabetes, or the disorder itself, and the health risks associated with diabetes. Preferably methods based on the use of readily available and inexpensively produced natural materials such as plants or plant parts. The methods should be both effective and inexpensive without introducing unwarranted toxicity and or mutagenic risks. There further remains a need to develop an effective method of extracting materials useful for treating diabetes or symptoms of diabetes from these plants or portions of these plants. The method of obtaining extracts from these plants should be convenient, and the extracts

should be safe and effective in modulating blood glucose levels. This leads to increasing demand for herbal product plants with antidiabetic activity and fewer side effects. And therefore the search, for new antidiabetic drugs still continues.

Accumulating evidence supports the hypothesis that several medicinal plants and phytochemicals are hypoglycaemic agents. Many plants have been reported to possess some form of antihyperglycaemic activity. Plant derivatives with hypoglycaemic properties have been used in folk medicine and traditional healing systems around the world, for example in East Indian, Mexican, Chinese (Covington, 2001) in Native American Indian and Jewish (Yaniv *et. al.*, 1987).

Natural products including plant materials of the neem, *Aloe* spp and *Urtica* spp have been used in traditional management of diabetes. Some studies have shown that the neem tree, *Azadirachta indica*, (Dixit *et al*, 1986) the stinging nettle, *Urtica* spp (Bnouham *et al*, 2003) and the *Aloe* spp (Ajabnoor, 1990) have hypoglycaemic effect on mammalian system.

Although these studies reveal that various preparations of these plants may have hypoglycaemic effect, most reports are purely speculative and only little data is available on such activity. This little data available is encouraging, however there is no systematic data to prove that these effects occur systematically after oral administration of these extracts and if these claimed effects occur, the dosage at which these effects occur has not been established. Data quantification is still vague and there is still insufficient evidence to draw definitive conclusions about the hypoglycaemic effect of their preparations. Despite several experimental studies that have been done on these three species, there is currently no pharmacological literature on their comparative hypoglycaemic effects.

Development of botanical antihyperglycaemic products has progressed in the recent years and such products are in the market today. In Kenya, examples of companies that sell them include Biop Herbal Therapy, Saroneem Biopesticides Ltd, Alternative Medicine and

Nature's Way Health. Unfortunately, such products are especially sold in their unextracted forms. In several countries some of these products are already registered.

Medicinal plants have evolved as an effective strategy to control the incidence of hyperglycaemia. Type II diabetes induced by Streptozotocin in Wistar rats which shows similarity to human diabetes is an ideal model for investigating the development of diabetes and the comparative effects of intervention by hypoglycaemic agents.

Preliminary studies revealed that the hydroalcoholic extract of the leaves of these plants possessed significant blood sugar lowering activity in normal glucose fed hyperglycaemic and Streptozotocin- induced diabetic rats (Chattopadhyay, 1993a). The purpose of this study is to prepare ethanol leaf extracts of *A. indica*, *U. dioica* and dried sap of *A. secundiflora* that have definable composition whose oral doses would be determined in the chosen animals and to compare the blood sugar lowering potential of these plants (leaf extract) against glibenclamide, a reference standard hypoglycaemic agent in normoglycaemic rabbits and streptozotocin-induced diabetic model rats. Hypoglycaemic effect will be assessed using plasma glucose concentration.

The ability to develop novel therapy of diabetes from traditional medicinal plants may be useful because if these plants are found to be effective they would go a long way to ease the burden of diabetes, since they are widely distributed in the third world countries in Africa. Therefore they would provide a cheap, available source of antihyperglycaemic agents, therefore counteracting diabetes morbidity, mortality that causes low production in terms of labour and high expenses leading to high poverty levels.

1.2 Blood sugar control

In the course of carbohydrate digestion, starch, which is a major constituent of food, sucrose and lactose are all hydrolyzed completely to monosaccharides. The resulting mixture of

glucose, fructose and galactose are absorbed into the hepatic portal blood and carried to the liver. Fructose and galactose are rapidly converted to glucose by enzymes in the liver, so that the sugar leaving the liver in the hepatic vein is normally all in the form of glucose. Thus, glucose is the form in which carbohydrates are transported in the blood and is distributed evenly between the erythrocytes and the plasma and can pass freely from one to the other. During normal daily living, blood glucose level is regulated by a homeostatic mechanism that efforts to maintain blood glucose concentration relatively at a constant (80 mg/100 ml of blood). This mechanism is mainly controlled by two hormones; insulin and glucagon produced by the islets of langer hans. Insulin is released by the beta cells when there is rising blood glucose levels. This is enhanced by several gastrointestinal tract hormones and elevated amino acid levels in the blood (Elaine and Marieb, 1999). Glucagon, on the other hand is produced by alpha cells when there is decreasing blood glucose levels. It creates the opposite effect, causing an increase in blood glucose by stimulating glycogenolysis in the liver (Elaine and Marieb, 1999).

Insulin increases glucose transport in fat and muscle cells by stimulating the translocation of the transporter GLUT4 from intracellular sites to the plasma membrane by increasing the rate of GLUT4 vesicle exocytosis and by slightly decreasing the rate of internalization (Pessin *et. al.*, 1999). GLUT4 is found in vesicles that continuously cycle from intracellular stores to the plasma membrane. High insulin level in blood promotes synthesis of triacylglycerides, uptake of branched chain amino acids by muscle, facilitating protein synthesis and inhibiting intracellular degradation of proteins; glycogenesis and suppresses gluconeogenesis by the liver (Elaine and Marieb, 1999). The final effect is reduction of blood glucose. In the event that insulin is absent or the regulatory mechanism is inhibited in one way or the other there's prolonged elevation in blood glucose levels, a clinical condition termed as *Diabetes mellitus*.

1.3 Definition of Diabetes mellitus

Diabetes mellitus is an endocrine disorder in which one passes large volumes of sweet urine. The large water loss leads to dehydration, unrelenting thirst and too much water taking, polydipsia. (Arky, 1979). *Diabetes mellitus* results from deficient insulin secretion or utilization at the target cell. Although there is normally large amount of glucose in dietary uptake, it is not available for appetite regulating cells at the feeding and satiety centres of the hypothalamus. A general feeling of great hunger for diabetics therefore results followed by excessive eating termed, polyphagia (Arky, 1979).

The blood glucose levels of a healthy man are 80 mg/dL on fasting and about 160 mg/dL in the postprandial state. Altogether there are three confirmatory *D. mellitus* test methods recommended by American Diabetes Association (ADA, 1997). First is the onset of classic symptoms- polyuria, increased thirst, unexplained weight loss and blurred vision. Secondly, a random plasma glucose of ≥ 200 mg/dL (11.1 mMol/L) and lastly a fasting plasma glucose of ≥ 126 mg/dL (7.0 mMol/L) after an overnight (at least 8h) fast, or a two-hour post load plasma glucose of ≥ 200 mg/dL (11.1 mMol/L) during a standard 75 g oral glucose tolerance test.

1.4 Types of Diabetes mellitus

With increasing rates of childhood and adult obesity, diabetes is likely to become even more prevalent over the coming decade (National Diabetes Fact Sheet, 1998). The incidence of *Diabetes mellitus* (DM) affects approximately 5% of the total population in the US and 3% of the population worldwide (WHO, 2002–2005).

There are four known subtypes of diabetes mellitus Type 1, Type II, gestational and other condition specific types, (The centers for disease control and prevention, 1998). Type I diabetes previously called insulin-dependent *Diabetes mellitus* (IDDM) or juvenile onset

diabetes comprise 10% of all *Diabetes mellitus* although its prevalence is increasing (Green and Eurodiab, 2001; ADA, Diabetes Care 2004)). It results from deficient insulin synthesis by the β cells of the pancreatic islets. The term insulin dependent means that the individual requires insulin to live. This type of diabetes afflicts a younger population with a peak age of around 14 years. In contrast, individuals with Type II diabetes have a normal or even higher level of insulin in their blood, but for some reason the insulin receptors are unable to respond to it, a phenomenon referred as insulin resistance (Elaine and Marieb, 1999). Previously, it was called non-insulin-dependent *Diabetes mellitus* (NIDDM) or adult-onset diabetes as it typically arises later in life, usually after the age of 40 years. Type II diabetes is increasingly common with age (Elaine and Marieb, 1999). Gestational diabetes develops in 2 to 5% of all pregnancies, but disappears postpartum. Risk factors include race or ethnicity and a family history of diabetes and obesity. Other specific types of diabetes result from specific genetic syndromes, surgery, drugs, malnutrition, infections and other illness, and accounts for 1 to 2% of all diagnosed cases of diabetes.

Type I diabetes is due to genetic and environmental factors. Inherited susceptibility to type I diabetes depends on several genes at different chromosome loci. The strongest linkage was found with the human leukocyte antigen (HLA)-D genes located within the major histocompatibility complex region on chromosome six. At least 20 other chromosomal regions may predispose a person to the development of type I diabetes. An environmental trigger is necessary to trigger the auto immunity (Redondo *et. al.*, 2001). This type of diabetes is a T-cell mediated autoimmune disease characterized by the destruction of insulin-secreting beta cells in the pancreatic islets of langerhans. Possible explanations as to what triggers the body's immune system, to turn on the organ itself include molecular mimicry, presence of an endogenous retroviral genome in diabetic islets, islet inflammation (insulinitis) and beta cell destruction induced by certain cytokines (Kukreja *et. al.*, 2001).

Type 1 diabetes can be divided into autoimmune or immune-mediated diabetes (Type 1A) and idiopathic diabetes with b-cell obstruction (Type 1B), (WHO, 1999 and the American Diabetics Association, 2001). As a result of the loss in beta-cell mass, severe insulin depletion leads to overt hyperglycaemia and a chronic disorder in carbohydrate, fat and protein metabolism. This becomes the genesis of many body complications that are often associated with diabetes. Untreated diabetes resembles that of chronic starvation and if acidosis rises are avoided, death results from tissue wasting (Elaine and Marieb, 1999).

Type II diabetes is generally recognized as a culmination of genetic and environmental risk factors. Genetic susceptibilities comprise one of the greatest risk factors (Weir, 1996). Frequent genetic risk factors include insulin resistance, decreased insulin secretion and post receptor defects. Each end of this spectrum has single gene disorders that affect the ability of the pancreatic β -cell to secrete insulin, (Fajans *et. al.*, 2001) or the ability of muscle, fat, and linear cells to respond to insulin action (Barroso *et. al.*, 1999). The main physiopathologic defects responsible for the development of hyperglycaemia are the continuous presence of insulin resistance and the progressive loss of β -cell function, which is the crucial defect in the development of type 2 diabetes.

Obesity, which is a major health problem, is the second most prevalent risk factor affecting more than 1 billion people worldwide (Imai, 2003). Other modifiable behavioral risk factors associated with insulin resistance include poor diet and lack of exercise (O'Dea, 1998), smoking (Merhnoosh *et. al.*, 1996) and stress (Kelly, 2000). Infections such as cancer of the pancreas, acute or chronic pancreatitis, cystic fibrosis and cushingism have also been sited as the other causes of this condition. Vast majority of people with type II diabetes have two metabolic characteristics; their β cells are defective in secreting insulin in response to elevating blood glucose or the target cells do not respond to an increase in insulin by elevating glucose transport (insulin resistance). The hyperglycaemic condition causes the

pancreas to respond by producing more insulin (hyperinsulinaemia) to compensate for the lack of cellular response to hyperglycaemia. Over time, the demand for insulin production due to increased insulin resistance leads to excessive stress on the beta cells which can lead to complete beta cell failure.

The loss of β -cell function is associated with the progression from normal glucose tolerance to impaired glucose tolerance and overt diabetes. Glucotoxicity hamper insulin synthesis by inhibiting the expression of insulin gene and inducing β -cell apoptosis and reduces the secretion of insulin through lowering β -cell sensitivity to glucose by hexosamine pathway. TNF- α inhibits insulin synthesis and secretion, induces β -cell apoptosis and may take part in the development of β -cell dysfunction in type 2 diabetes (Tsiotra *et. al.*, 2001). Poor insulin activity and chronically elevated blood insulin levels are associated with a number of serious conditions among these, hypertension and obesity (Zavaroni *et. al.*, 1994), ischaemic heart disease and dyslipidaemia.

1.5 Opportunistic ailments of diabetes

Through studies, it has been established that *Diabetes mellitus* predisposes patients to both opportunistic pathogens and ailments. Comparative study of urinary flora confirmed up to ten thousand colonies in young diabetics. This therefore is a potential source of urinary tract infection (Etzwiler 1960). Studies in diabetic patients have shown that fructose behavior causes sexual impotency (Benarroch, 1964). Ellenberg, (1964) also found that progress in diabetes causes neurogenic dysfunction. Studies of the gut undertaken by Honda *et. al.*, (1960) showed that 78% of the observed diabetics suffered from gastric atrophy.

1.6 Metabolic impairment resulting from Diabetes mellitus

Most of the metabolic diseases result from altered pathways. Diabetes is characterized by several of these impairments. Diabetics show a high protein catabolism rate. A-labeled albumin dose that takes 20-10 days to be depleted in normal individual may take only 4-11 days in diabetics. Polyunsaturated fatty acids metabolism in diabetics directly led to acidosis (Conan, 1994). However, elevation of total lipids for instance cholesterol, omega and beta lipoproteins causes hyperlipidemia in up to 66% of diabetic children (Lloyd *et.al*, 1962). Diabetics produce glycogen half as fast as normal individuals and due to this low rate most of the free glucose contributes to the hyperglycaemic status as well as being expelled in the urine (Kolata, 1979).

1.7 Vascular complications in diabetes

Long-term poor glycaemic control in diabetic patients leads to development of vascular and neural problems, microvascular neuropathy which affects the sensory and autonomic nerves, and can result in amputation of limbs and mortality. Retinopathy and results to significant vascular occlusion and nephropathy involves structural changes in the diabetic kidney such as overall increase in kidney size and glomerular volume, thickening of basement membrane, which is a characteristic of early nephropathy (Tsiliabary, 2003). This results in increased urinary albumin excretion culminating to proteinuria, hypertension, and tubular fibrosis (Osterby *et.al*, 2001) leading to decreased kidney function and progressive renal failure (Trevisian and Vibe, 2004). Macrovascular coronary artery disease (CAD) includes atherosclerosis in the carotid, cerebral and large arteries of the lower limbs, leading to heart attacks, heart failure and stroke complications due to glucose mediated vascular damage. Diabetes is documented as a leading risk factor (2-4 times higher) for CAD through

exacerbation of several risk factors associated with atherosclerosis including hypertension, dyslipidaemia, hyperinsulinaemia and insulin resistance.

1.8 Justification

Information dating back to the time of Ayurvedic medicine suggests infusions of plants are often used as herbal medications. No standardized dosage protocol has been developed for herbal extracts. Unfortunately in Kenya today there are, commodities in the market containing herbal products especially in unextracted forms recommended for human consumption without background data. Due to the variation in the amount of active compounds in different plants, it is worthwhile to systemize and establish data on different concentrations in order to formulate these substances for human use.

Even though studies on various preparations of *Aloe secundiflora*, *Urtica dioica* and *Azadirachta indica* on glycaemic control have been done, still there is inadequate information and data quantification is thus vague and therefore there is insufficient evidence to draw definite conclusions on the hypoglycaemic effect of these 3 plants (Yeh *et. al.*, 2003) and this warrants further studies. There is not yet sufficient evaluation of herbs for glucose control in diabetes (Yeh *et. al.*, 2003). WHO study groups emphasize strongly on the optimal rationale uses of traditional and natural indigenous medicines, (WHO 1994).

The information gathered from this study can be used to accurately relay to the Kenyan government the unexploited anti-hyperglycaemic value of these readily available and inexpensively produced natural plant products, unexploited pool of required dosage, their modification in terms of formulation and thus provide valuable information and a scientific basis for enhancing education and investment in safe herbal medicine.

1.9 Hypothesis

Ethanol leaf extracts (80 mg/kg b.w.) of *Urtica dioica*, *Azadirachta indica* and dried *Aloe secundiflora* sap have no hypoglycaemic activity.

1.10 Aims and objectives of the study

This study was undertaken with a view of generating basic information on comparative hypoglycaemic potential of *Aloe secundiflora*, *Urtica dioica* and *Azadirachta indica*, using rabbit and diabetic rats.

The study involved two stages; extraction and formulation of crude extracts, and the evaluation of the effects of these three plants against hypoglycaemic formulation. The specific objectives of the study were:

1. Extraction of biological activity in the leaves of *Aloe secundiflora*, *Urtica dioica* and *Azadirachta indica* and formulate a syrup for oral administration to rats and normoglycaemic rabbits.
2. To investigate the hypoglycaemic activity of the plant extracts in diabetic rats and normoglycaemic rabbits, and compare with glibenclamide a standard drug.

CHAPTER TWO

LITERATURE REVIEW

2.1 Progress in Diabetes mellitus therapy management

Tremendous advances in our understanding of the pathogenesis and the treatment of *diabetes mellitus* have been witnessed since the discovery of insulin by Frederick Banting and Best in 1921 (Rosenfeld, 2002). To date, insulin therapy from highly purified recombinant human insulin preparations remains the only treatment proven safe and effective after clinically significant beta-cell destruction takes place. It is true that type I diabetic individuals require lifelong injections of insulin for survival (Rossini *et. al.*, 2003). Portable insulin infusion pumps are also available but not all patients can afford the device. Inhaled insulin containing mist and powder are in clinical trials but not yet available.

Therapies for type 2 diabetes are limited. Treatment goals for type 2 diabetes patients specify targets for glycaemia and other cardiovascular risk factors (ADA, 2005). Long-term efficacy of lifestyle intervention is often poor. The risk of diabetic complications is closely related to prevailing level and duration of hyperglycaemia. Lifestyle interventions irrespective of the concomitant use of pharmacological anti-diabetic therapy remain the cornerstone of management of type 2 diabetes (International Diabetes Federation, 2005). Overweight individuals with impaired glucose tolerance are encouraged to loose weight, adhere to recommended diets and exercise regularly, as weight loss and muscle gain delay the onset of type II diabetes.

Oral pharmacological agents that lower overall blood glucose level using different mechanisms in type II diabetes have been developed. This include, insulin secretagogues which stimulate glucose metabolism, and the glucose sensor (glucokinase) of GLUT2 (glucose transporter) their examples include sulfonylureas and meglitinides. Sulphonylureas and the short-acting insulin releaser (repaglinide) act directly on the islet b cells to close ATP-sensitive K⁺ channels, which stimulate insulin secretion (Groop, 1992). There are also basal hepatic glucose production suppressors such as metformin. Metformin is the only antidiabetic drug that counters insulin resistance. Its glucose-lowering effect is mainly a consequence of reduced hepatic glucose output (Gluconeogenesis and glycogenolysis) and increased insulin-stimulated glucose uptake and glycogenesis in skeletal muscle (Bailey and Turner, 1996). Muscle and liver insulin sensitizers like thiazolidinediones (Furnsinn and Waldhausl, 2002) are also in use. ligand-activated transcription factors with markedly enhanced potency and selectivity for the receptor have been discovered and are also in use (Moller and Greene, 2001). Other oral pharmacological agents include α glucosidase inhibitors. These compounds delay the intraluminal production of monosaccharide, particularly glucose (Creutzfeldt, 1988). Acarbose competitively inhibits α -glucosidases that are associated with the brush border membrane of the small intestine and thus inhibits the digestion of complex polysaccharides and sucrose. (Lebovitz, 1997). Blocking the enzyme degradation of complex carbohydrates in the small intestine, improves glycaemic control. However the effectiveness of many oral pharmacological glucose-lowering agents is temporary and within 4 to 5 yr of therapy can no longer compensate for beta-cell failure.

The first option for complicated long-standing type I and type II diabetes mellitus patients is cell transplant therapy. This field has witnessed pancreatic islet transplants (PIT) (Shapiro *et. al.*, 2000). Major obstacles to overcome include the need for chronic immuno suppression and the shortage of human organs for transplantation (Zwillich, 2000). This is

coupled by the vulnerability of pancreatic islets to instant blood inflammatory reaction after transplantation, poor vascularization and exposure to diabetogenic immunosuppressive drugs (Paraskevass *et. al.*,2000).

An alternative method, gene therapy is in the offing. Researchers still strive to exploit the increased ability for environmental manipulation and discovery of innovative genetic and genomic tools in animal research to unravel the complex causes of autoimmunity. Further, to explore new strategies, and hope-fully to prevent the disease one day.

Research on oral herbal therapy forms of medication is blossoming and this follows noted use of some plants as traditional anti-diabetes concoctions. Plant derivatives with purported hypoglycaemic properties have been used in folk medicine and traditional healing systems around the world e.g. Native American Indian, Jewish (Yaniv *et. al.*, 1987), Chinese (Covington, 2001), East Indian and Mexican. Some of these plants are still in local use by different societies throughout the world. Many modern pharmaceuticals used in conventional medicine today also have natural plant origins. Among them is metformin which was derived from the flowering plant, *Galega officinalis* (Goat's Rue or French Lilac), which was a common traditional remedy for diabetes (Oubre *et. al.*, 1997). Similarly, the use of vitamin and mineral supplements for primary or secondary disease prevention is of increasing interest (O'Connell, 2001).

2.2 Oral herbal therapy

Approximately 80% of the population in developing countries relies on traditional medicine for their health care (Namjoshi, 1982). This is supported by the fact that the earliest records that plants were used as medicine date from Neanderthal burial sites dug in Iraq 60,000 years ago. The Chinese, Great Herbal dates back from 3000 B.C., while the beginnings of Western Herbarium in the Middle East date from 2,500 B.C. The Ebers papyrus parchment lists both individual herbs and prescriptions for specific diseases from 1500 B.C. Ancient

Greek physician Hippocrates has been adopted as the father of medicine. The bible itself lists over forty plant medicines including frankincense and myrrh.

Traditional knowledge on thousands of useful herbs has been reported. Ethnobotanical studies of traditional herbal remedies used for diabetes around the world have identified more than 1,200 species of plants with hypoglycemic activity. Marles and Farnsworth, (1995) observed that these plants are broadly distributed throughout 725 different genera. These plants have generated extraordinary interest in recent years as potential sources of diabetes control agents. The pharmacopoeia of India is especially rich in herbal treatments for diabetes. Studies have shown that eighty-five percent of the 20 antidiabetic plants most widely used around the world are prescribed in India (Marles and Farnsworth, 1995). The first hypoglycaemic compounds to be used by man were from plants, the biological activities of which were known from the earliest recorded times.

In the Asian continent certain plants have a long history of use as hypoglycaemic agents in Ayurveda. These include Ginseng species, *Ocimum sanctum*, *Coccinia indica*, *Trigonella foenum graecum*, *Gymnema sylvestre*, *Momordica charantia* and the *Allium spp.* Roots of certain plants in the ginseng species have shown hypoglycaemic in streptozotocin rat models (Shapiro and Gong, 2002). Ginseng species leads to decreased rate of carbohydrate absorption into the portal hepatic circulation, increased glucose transport and uptake mediated by nitric oxide, increased glycogen storage and modulation of insulin secretion (Shane-Mc, 2001). Biological activity of these roots has been attributed to the presence of triterpenoid saponin glycosides (ginsenosides or panaxosides). It is postulated that *Ocimum sanctum*, (holy basil) enhances cell function and insulin secretion and this may explain its hypoglycaemic properties. Studies in animal models support these effects (Chattopadhyay, 1993a). Available literature also suggests that *Coccinia indica* herb may have insulin-mimetic properties (Kamble *et. al.*, 1998). Defatted seeds of fenugreek (*Trigonella foenum*

graecum) a legume herb rich in fiber, saponins, and protein has been documented to delay gastric emptying, slow carbohydrate absorption and inhibit glucose transport, as well as increase erythrocyte insulin receptors and modulate peripheral glucose utilization. It may also modulate exocrine pancreatic secretion of insulin (Sharma, 1986). Other studies show that *Gymnema sylvestre*, another commonly used Ayurvedic herb may lead to regeneration of islets of Langerhans, decreases blood glucose and increases serum insulin (Shanmugasundaram *et. al*, 1990).

Some albeit limited data also suggests a potential effect of *Momordica charantia* in diabetes control. A vegetable indigenous to tropical areas, its active components are thought to be charantin, vicine, and polypeptide-p (an unidentified insulin-like protein similar to bovine insulin) (Marles and Farnsworth, 1995; Zafar and Neeja, 1991). *M. charantia* increases insulin secretion, tissue glucose uptake, liver muscle glycogen synthesis, glucose oxidation, and decreases hepatic gluconeogenesis. Studies in alloxan-induced diabetic rabbits have suggested it's hypoglycaemic effects (Marles and Farnsworth, 1995; Zafar and Neeja, 1991).

Most commonly used worldwide for flavourful cooking, the *Allium spp* of the lily family *Allium sativum* (garlic), and *Allium cepum* (onion) are known to have hypoglycaemic properties. Garlic has a potential antioxidant activity and microcirculatory effects. *Allium spp* has shown increased secretion or slowed degradation of insulin, increased glutathione peroxidase activity and improved liver glycogen storage (Shane-Mc, 2001). *Silibum marianum* (milk thistle), a member of the aster family, rich in flavonoids and potent antioxidants has a similar mechanism. Even in non-diabetic individuals *Allium sativum* (garlic) shows significant decreases in fasting serum glucose (Keisewetter *et. al.*, 1991).

Payne (2001), reported an increase in use of complementary and alternative medicine (CAM) among the general public. In response to this, the American Diabetes Association

issued a position statement in 2001 on 'Unproven Therapies' encouraging health care providers to ask their patients about alternative therapies and practices, evaluate each therapy's effectiveness, be cognizant of any potential harm to patients, and acknowledge circumstances in which new and innovative diagnostic or therapeutic measures might be provided to patients. In view of this, Weiger *et. al.*, (2002) reported that no herb or supplement has sufficient evidence to actively recommend or discourage its use among patients with diabetes.

In Kenya, the ratios of healthcare to population vary from 1:200,000 to 50,000 against the government's target of 1:20,000 and less (Sindiga, 1995a). Odera, (1997) reported that over 70% of the Kenyan population relies on traditional medicine as their primary source of health care, while over 90% have used medicinal plants at one time or another. In this respect, traditional medicines play a vital role in the well being and development of rural people in Kenya. However, despite the fact that a great percentage of Kenyan population relies on herbal medicines and in spite of the prominent role that traditional medicine plays in Kenya, it is the least understood of all medical systems (Sindiga, 1995a), and yet very few Kenyan indigenous plants have been screened for their pharmaceutical potential despite of their richness in chemicals for pharmaceutical industry (Kokwaro, 1993).

2.3 *Aloe secundiflora*

The name 'aloe' is derived from the Arabic word 'alloeh' meaning a shining bitter substance. Few plants can claim a 4,000-year history of use for essentially a wide range of purposes like aloe. *A. secundiflora* is a desert plant resembling the cactus. It belongs to the Liliaceae family. The herb is a succulent, almost sessile perennial herb growing up to a height of 0.06m to 1.2m with the average of around 0.7 to 0.9m in length. Each plant has 12-16 leaves that may weigh up to 1.3kg when mature. The plants can be harvested every 6

to 8 weeks by removing 3 to 4 leaves per plant. Aloe's thick, fleshy, tapered, and strongly cuticularized spiny leaves grow from a short stalk base near the ground level in a rosette pattern. They can be up to 10cm broad. The plant cannot tolerate temperature much below 4°C. Aloe has bright yellow tubular flowers 25 – 35cm in length arranged in slender loose spike stamens frequently project beyond perianth tube. The fruit is a triangular capsule containing numerous seeds that are produced from same root structure year after year. In certain parts of United States, *Aloe vera* plant is now commercially produced.

Aloe spp belongs to cites Appendix II (the Convention on International Trade in Endangered Species of Wild Fauna and Flora). These are species that, although not threatened with extinction now, might become so unless trade in them is strictly controlled and monitored. The aloe juice should not be confused with the gel. The aloe juice from which the drug used in this study was prepared consists of the dried solidified juice originating in the large pericycle cells and adjacent leaf parenchyma (Wallis, 1985), and flowing spontaneously from the cut leaf and allowed to dry with or without the aid of heat. Liquid gel on the other hand is the bitter yellow exudate originating from the bundle sheath cells of the fresh *Aloe Vera* leaf. *Aloe* spp grows in Mediterranean region, West Indies and in Africa where, it is widely spread in Sahelian zone and in East Africa (Trease and Evans, 1978).

Aloe contains a wide variety of chemical constituents. These include, anthraquinones (Aloes contain C glycosides and resins such as emodin, antranol), anti-microbials (cinnamonic acid, phenol lupeal, lectins), carbohydrates and lipids. Aloe also contains minerals, vitamins, enzymes and amino acids. The fundamentals of it's carbohydrates are called mucopolysaccharides. Gowda (1979), demonstrated that mannose phosphate is the significant constituent in the 50% *Aloe vera* extract.

Uses described in folk traditional record medicine include treatment of skin diseases such as acne and seborrhoeic dermatitis haemorrhoids, anaemia, glaucoma, petit ulcer, tuberculosis, blindness, dental disorder, fungal infections and as a natural remedy for burns. Preliminary data suggests *Aloe Vera*'s potential effect in glycaemic control. Various parts of the plant have been used for similar purposes. Reports in animal models have been inconsistent (Yongchaiyudha *et. al.*, 1996; Ghannam *et. al.*, 1986; Ajabnoor, 1990). Studies in both type I and types II *Diabetes mellitus* in animal models reflect hypoglycaemic activity of various Aloe constituents (Blitz *et. al.*, 1963). Glibenclamide and aloes have been shown to induce hypoglycaemia in normal mice, (Ghannam *et. al.*, 1986) in alloxan diabetic mice (Ajabnoor, 1990; Ghannam *et. al.*, 1986) and in streptozotocin – induced mice (Rajasekaran *et al*, 2004).

Ghannam *et. al.*, (1986) reported that dried sap of aloe plant (aloes) reduced blood glucose level in patients with non-insulin dependent diabetes with no change in body weight. Okyar *et. al.*, (2001) found that pulps of *Aloe vera* leaves devoid of the gel could be useful in the treatment of non-insulin dependent *Diabetes mellitus*. It's hypoglycaemic activity has been attributed to a group of polysaccharide fractions known as glycans, first found in *Aloe aborescens* variety, in natalensis leaves (Solov'era, 1958). Arborans A and B were later isolated by Hikino *et. al.*, (1986). Morgan (1987) showed that the mannose phosphate binds to the insulin like growth factor receptor. Rajasekaran *et. al.*, (2004) reported that *Aloe vera* extract maintains the glucose homeostasis by controlling the carbohydrate metabolizing enzymes. Data on the effect of *Aloe vera* on blood glucose levels of normal and diabetic animal models are scanty.

Constituents of aloe spp have been reported to have other strong biological and medicinal effects in modern scientific literature. Chitosan, associated with Aloe, possesses a lipid lowering activity, which prevents atherogenic processes, (Geremias *et. al.*, 2006), and may

prevent atheromatous heart disease (Agarwal, 1985). Aloe-emodin has anti-neoplastic activity against some human cancer cell lines (Yu *et. al.*, 2006), inducing DNA damage through generation of reactive oxygen species in these cells from the prooxidant (Lee 2006 *et. al.*). The triterpene, lupeol is a possible ulceroprotective agent (Agarwal, 1985) being used in the treatment of gastric and duodenal ulcers (Hulter *et. al.*, 1996). Other studies also show that Aloe possesses an anti-inflammatory activity (Gupta *et. al.*, 1981). Its gel extract contains enzymes with an inhibitory action on the arachidonic acid pathway (Gupta *et. al.*, 1981). Its active healing principle exists in the anthranols, and mucopolysaccharides. A glycoprotein, Aloctin A, isolated from *Aloe arborescens* Mill, inhibits adjuvant arthritis in rats and carrageenin-induced edema in rats (Saito, 1982).

Aloe exhibits immunomodulatory activity by guarding against damage by free radicals and unwanted toxins in the body. *Aloe vera* shows antioxidant activity against superoxide and hydroxyl radicals (Zhang *et. al.*, 2006). One of the two dihydrocoumarin derivatives isolated from Aloe, increases the phagocytic activity and stimulates the production of superoxide anions (Zhang *et. al.*, 2006). On the other hand, one of the lectins has mitogenic activity on lymphocytes. Certain studies indicate that aloe could be harmful to pregnant women or people with haemorrhoids or irritable bowel syndrome (Medicinal plants of Nepal, 1993). Genotoxicity studies show aloe-containing laxatives pose cancer risk to humans when used as directed. Present as minor components in senna, two hydroxyanthraquinones (HAs), aloe-emodin and emodin, might represent a genotoxic or cancerogenetic risk for man (Grimminger and Witthohn, 1993).

2.4 *Azadirachta indica*.

Azadirachta indica has been long revered for its many healing properties. It is commonly known as “Muarobaine” in Swahili, the word “Muarobaine” meaning forty implying that

the plant cures forty diseases. It is part of almost every aspect of life in many parts of the Indian sub continental, up and including even the modern era (Astangahrdaya Samhita, 1941). The first indication that neem was being used as a medical plant was about 4,500 years ago. This was the high point of the Indian Merappa culture one of the greatest civilization of the ancient world. Neem has been used for medicinal, agricultural, industrial and commercial exploitation.

A. indica A. Juss., synonyms *Melia azadirachta* L. and *Melia indica* (A. Juss) brand belongs to the Meliaceae family. It is a hardy, fast-growing evergreen tree with a straight trunk, long spreading branches moderately thick bark and round crown. Mature trees attain up to 10m, with fruit bearing starting at 4 – 5 years, fully productive within 10 years and the plant may live for more than 200 years (Ahmed and Grainge 1986). *A. indica* is endemic in the indo-pakistan sub-continent. It grows in the South Pacific islands and in the Middle East. In Africa, it's widespread in the Sahelian Zone, in East and West Africa (Ahmed and Grainge, 1986). It grows in Cuba and Central America (Nicaragua) as a plantation tree (Lewis and Elvin Lewis, 1983).

A. indica was first described as *Melia azadirachta* by Linnaeus in his species plantarum (1753). De Jussieu (1830) was the first to separate *Azadirachta* from *Melia*. It is considered to belong to the Melieae, one of the five tribes of the subfamily Melioideae within the Meliaceae family. Taxonomically, it belongs to order-Rutales, suborder-Rutinae, family-Meliaceae, subfamily-Melioideae, tribe-Melieae, genus-*Azadirachta*, and species-*indica* (Meeuse, 1983; Hegnauer, 1983). The chemical constituents reported include terpenes, steroids, and limonoids- (Devakumar and Mukerjee, 1985). Salannin, phenolics- flavonoids flavonolglycosides, tannins, coumarins and dihydrochalcones (Van der Nat *et. al.*, 1991). Carbohydrates and proteins (Mukherjee and Srivastava 1955; Anderson and Hendrie 1971); sulphurous compounds in the seeds and cyclic tri- and tetrasulphides in the leaves;

alkaloids present in the bark, in the fruits and in the leaves. Nimboctin acids found in the leaves, the seed oil from the seeds contain saturated fatty acids among others (Van der Nat *et. al.*, 1991).

Data on hypoglycaemic activities of neem is varied. Luscombe and Taha, (1974) observed that aqueous leaf extract of neem results to marked hypoglycaemia in normoglycaemic animals. However, no effects were seen in totally pancreatectomized and alloxan-induced hyperglycaemic animals. In experiments involving diabetic patients, leaf paste reduced daily insulin dosages (Shukla *et. al.*, 1973), even though reduced dosages of insulin alone were incapable of producing the same effect when given without leaf preparations. Seed oil exerts a similar effect. When given orally, its extracts reduce blood sugar levels in both normoglycaemic and alloxan –induced hyperglycaemia, (Dixit *et. al.*, 1986; Kholsa *et. al.*, 2000). In a clinical trial involving eighty-five diabetics aged over 35 years Bhargava *et. al.*, (1985) observed a significant fall in both fasting and postprandial blood sugars and the insulin or oral requirements fell by 25 to 50%.

Nimbidin, a constituent of the neem plant exerts significant effect on blood glucose levels either by release of endogenous insulin in a way similar to the effects of sulfonylureas (Luscombe and Taha, 1974; Shukla *et. al.*, 1973), or by increased peripheral glucose utilization, enhanced insulin release and decreased glucose reabsorption at the proximal tubules (Dixit *et al.*, 1986). Studies suggest that *A. indica* prevents or helps delay *Diabetes mellitus* (Kholsa *et. al.*, 2000) as pretreating rabbits with seed oil and leaf extract prior to alloxan administration, partially prevents the rise in glucose level. Jacobson (1995), observed that oral administration of neem oil at 200 mg /rat produces severe hypoglycaemic effect.

Biological activities of its preparations are enormous. Certain activities of the plant's extracts interfere with hormone production and inhibit feeding in a wide variety of insect

species and nematodes. Rembold, (1985) reported that azadirachtin is primarily the active agent that interferes with the synthesis of both the sesquiterpene juvenile hormones and the steroid hormones (ecdysteroids) responsible for the moulting of insects, thereby disrupting growth. Catechin possesses anti-inflammatory properties (Fujiwara *et. al.*, 1984a, b; Vander Nat *et. al.*, 1991). Nimbidin shows anti-histaminic properties (Pillai and Santhakumari, 1984a) by directly interfering with the immune system (Van der Nat *et. al.*, 1991) and antipyretic activity (Okpanyi and Ezeukwu, 1981). The seed oil of *A. indica* is antifungal (Bhowmick and Choudhary, 1982). Leaf extracts of *A. indica* have antiviral properties (Rai and Sethi, 1972). Nimbolide and Mahmoodin show anti-bacterial activity (Rojanapo *et. al.*, 1985; Devakumar and Sukh Dev, 1996). Purified neem's seed fractions inhibit growth and development of human malarial parasite, *P. falciparum* (Dhar *et. al.*, 1998), making it effective against both chloroquin-resistant and sensitive strains (Khahid *et. al.*, 1989).

Isolated bark polysaccharide Gla and Glb show antitumour effects, (Fujiwara *et. al.*, 1982). Okpanyi and Ezeukulu (1981), also reported that neem's limonoids and polysaccharides show anti-inflammatory, anti-rheumatic and anti-arthritic activity. Nimbidin prevents mucus depletion and cell degranulation (Garg *et. al.*, 1993), thus producing a protective effect against certain induced gastric and duodenal lesions in rats and guinea pigs respectively (Pillai and Santhakumari, 1984a). Other studies show that leaf extracts of *A. indica* nimbidin included, suppress CNS activity (Pillai & Santhakumari, 1984b). In rabbits, neem extract induces a profound and dose dependent hypotension. With a dosage of 40 mg/kg i.v. exhibiting antiarrhythmic activity against quabain -induced dysrhythmia (Van der Nat *et. al.*, 1991). *A. indica* prevents and terminates pregnancy in rats (Tewari *et. al.*, 1986). In monkeys and women when used intra-vaginally, and in male mice, it shows anti-fertility properties (Deshpande *et al.* 1980). This could be a non hormonal mechanism,

mediated through its spermicidal effects and may have fewer side effects than steroidal contraceptives.

In acute toxicology studies involving mice, methanolic extract of neem bark demonstrated oral $L_D 50$ at about 13g /kg (Kanungo, 1996). Aqueous extract of neem bark (2g /kg b.w.) given orally shows no apparent change in weight, hematological parameters, enzyme levels and histopathology of several organs and shows no lethal effect (Project, SSP-60, 1999). Gandhi *et. al.*, (1988) reported that neem oil shows toxicity of CNS and the lungs in rats and rabbits with $L_D 50$ of 14 ml / kg and 24 ml/kg respectively. In humans, intoxication produces diarrhoea, nausea, vomiting, acidosis, and encephalopathy in several isolated cases.

2.5 *Urtica dioica*

Greek physicians Dioscorides (first century Common Era (C.E.)) and Galen (Circa (Ca.) 130- 200 C.E) reported nettle leaf had diuretic and laxative action and was useful for asthma, pleurosy and for treatment of spleen –related illness. In Africa, India and North America the herb is used for nosebleeds, excessive menstruation, to treat internal bleeding and on burns (List and rhammer, 1979). It's an antirheumatic drug and also used as a gynaecological aid during labour (Moerman, 1998). Products and preparations of the nettle are used as growth stimulant in garden plants, blood purifiers and also as insect repellent. Their twines are used to make sails, and their fibres cloth. Cooked nettle leaves are eaten as vegetables.

U. dioica belongs to the urticaceace family. In English it is named nettle or common nettle, net plant or devils leaf. Generally it is an erect perennial herb, 50-300cm tall with four sided stems armed with stinging hairs. They are strongly bristly and form long spreading rhizomes usually in colonies. Its leaves are oppositely arranged on the stem and range from

7-15 cm long. The leaf blades could be narrowly lanceolate rounder or pointed at base to broadly ovate and often ovate at base coarsely sharp toothed. It's numerous inconspicuous flowers sometimes green or tinged pink in colour, hang in form of clusters from the upper leaf axils, with four tiny sepals and no petals. It is a dioecious plant, the male and female flowers being on separate plants. Fruits are achenes, lens shaped and flattened, 1.5mm long and enclosed by the inner sepals. The "stinging hairs" have very sharp spines, which are hollow and arise from a swollen base, which instantly releases an acid when the hair tip pierces the skin. This acid causes itching or and burning for a few minutes to a couple of days.

The plant belongs to the division magnoliophyta, class magnoliopsida, order Rosales, Family urticaceae, genus *Urtica* and species *U. dioica*. The genus name *Urtica* comes from the latin verb 'urere' meaning 'to burn' because of its urticate (stinging) hairs. The species name *dioica* means "two houses" because the plant usually has either male or female flowers (Bombardelli and morazzoni, 1997). Stinging nettle herb grows wild throughout the temperature zones of both hemispheres worldwide.

Plant constituents include; Acids (carbonic, caffeic, cafffeoylmalic, chlorogenic, formic, silicic, citric, fumaric glyceric, malic, oxalic, phosphoric, quinic, succinic, threonic and threono 1, 4- lactone) (Bakke *et. al.*, 1978). Amines (acetylcholine, betaine, choline, lecithin, histamine, serotonin) (Adamski and Bieganska, 1984), and a glycoprotein (Andersen and Wold, 1978). Flavonoids such as flavonol glycosides (Chaurarasia and Witchl, 1987) and upto 20% minerals including calcium, potassium, silicon, lignans and other constituents.

Research suggests that nettle's anti-inflammatory actions are attributed to its ability to interrupt the production and actions of inflammation producing-immune cells such as cytokines, prostaglandins and leukotreines in the body, (Obertreis *et. al.*, 1996).

Nettle herb juice shows minor decrease in body weights and systolic blood pressure. The treatment produces a distinct diuretic effect (Kirchhoff, 1983; Varro, 1994). Nettle extracts performs better than the control drug (furosemide). Legssyer *et. al.*, (2002) reported that nettle extracts reduces heart rate, and increases urine output and sodium excretion. It induces a strong bradycardia accounting for its hypotensive action. (Abdelkhaleq *et. al.*, 2002). *Urtica dioica* alleviates fluid retention in men who suffer from benign prostatic hyperplasia (BPH). High potassium ion concentration in nettle aqueous decoctions contributes to their diuretic activity. Szentmihályi *et al.*, (1998) reported that it's lectin, binds to the human sex- hormone binding globulin (SHBG) preventing the latter from binding to its receptors on the sex glands thus regulating plasma levels of free steroids. Alcoholic extract of nettle root is also reported to improve bladder outlet obstruction symptoms and decreases post-voiding residual urine (Schneider *et. al.*, 1995). In Germany, stinging nettle herb is licensed for diuretic action, treatment of rheumatic ailments, irrigation therapy in inflammatory of the lower urinary track and treatment for urticaria, herps, eczema, hypersensitive reactions in the skin, joints and burns (List and rhammer, 1979). In the United States it is prescribed by the naturopathic physicians and licensed acupuncturists as a component in formulas used to treat hayfever and other allergies.

The effects of *U. dioica* on normal and streptozotocin induced diabetic mice have been examined. Although, Swanston-flatt *et. al.*, (1989) found that infusions of the plants aggravate the diabetic condition in streptozotocin induced diabetic mice and in dextrose induced diabetic mice (Roman *et. al.*, 1992), its ethanol and aqueous extracts of nettle were observed to lower hyperglycaemia in the same animal models (Neef *et. al.*, 1995). Bnouham *et. al.*, (2003) also reported the anti-hyperglycaemic activity of aqueous extract of *U. dioica*. However, nettle did not show hypoglycaemic effect in alloxan-induced diabetic rats.

Oliver and Zahland (1979) reported that a hypoglycaemic component, urticin, in nettle is responsible for lowering the blood sugar concentration in hyperglycaemic rabbits. Also isolated from *Urtica pilulifera* L. seeds another compound, a lectin, was reported to increase either the pancreatic secretion of insulin from beta-cells of islets or release the insulin from the bound insulin, thus exerting a hypoglycaemic effect (Kavalali *et. al.*, 2003). Another active ingredient fraction, F(1) isolated from *U. dioica* leaves has been shown to cause a marked increase in insulin secretion (Farzami *et. al.*, 2003). The component F (1) increases insulin content of blood sera in normal and streptozotocin diabetic rats by six times. Other studies suggest that *U. dioica* has a prophylactic effect and shows a potent alpha-glucosidase inhibitor activity (Onal *et. al.*, 2005).

Stinging nettle is generally considered safe when used as directed. Occasional side effects reported include mild gastrointestinal upsets, fluid retention, skin rash when handling, secondary to the noted stinging (Blumenthal *et. al.*, 1998). Illarionova *et. al.* (1995) observed that consumption of nettle tea causes gastric irritation, a burning sensation of the skin, oedema and also oliguria. On toxicity studies in rats and mice Tita *et. al.*, (1993) found that ethanol extracts of nettle show low toxicity after oral and intraperitoneal administration at doses equivalent to 2 g/kg. Nettle may interact with concurrent therapy for diabetes, and high or low blood pressure. Betaine and serotonin constituents of nettle affect the menstrual cycle and are abortifacient. Excessive use should be avoided during lactation. Oral administration of nettle extract up to 250 mg/kg is reported to be devoid of antifertility activity in mice.

2.6 Extraction solvent

Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Although water is almost the universal solvent used to extract activity, it does not yield more than 10% active ingredient without high heat. The heat destroys the active ingredients. Organic solvents such as ethanol, acetone, and methanol are also often used to extract bioactive compounds (Eloff, 1998). Each compound being extracted has different solubility in different solvents, and the optimum is not known. Properties of a good solvent in plant extractions includes, low toxicity, ease of evaporation at low heat, promotion of rapid physiologic absorption of the extract, preservative action and the inability to cause the extract to complex or dissociate. Conventionally, efficiency of ethanolic compound with water is usually 5 times. Methanol is more polar than ethanol but it interferes with the nervous system and due to its cytotoxic nature, it is unsuitable for extraction as it may yield incorrect results.

Ethanol, however, is the most commonly used organic solvent by herbal medicine manufacturers because the finished products can be safely used internally by consumers of herbal extracts (Low Dog, 2009). Due to the variation in composition of active compounds, different plants may require different concentrations of ethanol to achieve maximum recovery of bioactive components. No standardized extraction protocol has been developed for preparation of herbal extracts, but 20-95% of ethanol-water mixture is frequently used by the herbal medicine industry to prepare ethanolic extracts (Ganora, 2008). Ethanol easily penetrates the cellular membrane to extract the intracellular ingredients from the plant material (Wang, 2010) and 60-70% ethanol, detects higher concentrations of more bioactive flavonoid compounds due to its higher polarity than pure ethanol. By adding water to the pure ethanol up to 30% for preparing 70% ethanol,

the polarity of solvent is increased (Bimakr, 2010). In this work however, 60% ethanol was used to extract the bioactive compounds.

2.7 Formulation Additives

A combination of reduced pH with weak organic acids and reduced water activity is the key element in the stability of many shelf stable foods (Presser *et. al.*, 1998). Carboxymethyl-cellulose (CMC) is a salt of Sodium which is physiologically an inert cellulose ether polymer, with improved compatibility with other soluble components. It is generally recognized as safe (GRAS) (U.S. Code of Federal Regulations, 1745). It is often used to suspend, thicken, stabilize, gel, or otherwise modify the flow characteristics of aqueous solutions or suspensions.

Solutions of CMC are however not immune to attack by cellulases (hydrolytic, viscosity-destroying enzymes) and chemical degradation. However, it is more resistant to microbiological attack than many other water-soluble gums. It is stable in several organic acids including Citric acid. This gives optimum pH medium for the working of Sodium Benzoate. Sodium Benzoate 0.1 % (W/V) an antifungal agent (Bosund, 1962) prevents the growth of fungal moulds by increasingly making the cytoplasm acidic (Lambert and Stratford, 1999). This acidification of the cytoplasm may prevent growth by inhibition of glycolysis (Hans *et. al.*, 1983), prevention of active transport (Freese *et. al.*, 1973) or by interference with signal transduction and in consequence restricting growth of micro-organism. Sodium Benzoate is GRAS additive (U.S. Code of Federal Regulations, 1745). Daily consumption of saccharin, a natural sweetener, below the acceptable daily intake (ADI) levels had no significant effect on blood glucose concentration or blood lipids in people with diabetes (Cooper *et. al.*, 1988). Saccharin is absorbed and excreted in the urine, although in itself it is not metabolized by the body (Renwick, 1986).

2.8 Animal models for type 2 Diabetes

Rattus norvegicus was the first mammalian species to be domesticated for scientific research, with work dating to before 1828 (Hedrich, 2000). The first recorded breeding colony for rats was established in 1856 (Hedrich, 2000). The role of the rat in medicine has transformed to indispensable tool in experimental medicine and drug development especially in human medical research in diabetes (Ravingerova *et. al.*, 2003). Almost all human genes known to be associated with diseases have counterparts in the rat genome and appear highly conserved through mammalian evolution, confirming that the rat is an excellent model for many areas of medical research.

The animal models of type 2 diabetes can be obtained either spontaneously or induced by chemicals or dietary or surgical manipulations and or by combination. Large number of new genetically modified animal models including transgenic, generalized knock-out and tissue-specific knockout mice have been engineered for the study of diabetes. Frequently used chemically-induced rat models include alloxan and streptozotocin rats. Wistar rats are an outbred strain of albino rats belonging to the species *Rattus norvegicus*. Developed at the Wistar Institute for use in biological and medical research, it was notably the first rat strain to serve as a model organism at a time when laboratories primarily used *Mus musculus*, the common house mouse. Currently it is one of the most popular rat strains used for laboratory research. The Wistar rat is characterized by its wide head, long ears, and a tail length that is always less than its body length.

The medium size, very docile nature of the New Zealand white rabbits makes them particularly easy to maintain, handle and restrain. While their white large unpigmented ears facilitate repeated venous injections and bleeding. Rabbit physiology is fairly similar to that of humans so provides very useful models in many different areas of biomedical research

including diabetes. New Zealand white belong to order Lagomorpha, together with hares, pikas and American cottontail rabbits.

2.9 Streptozotocin (STZ)

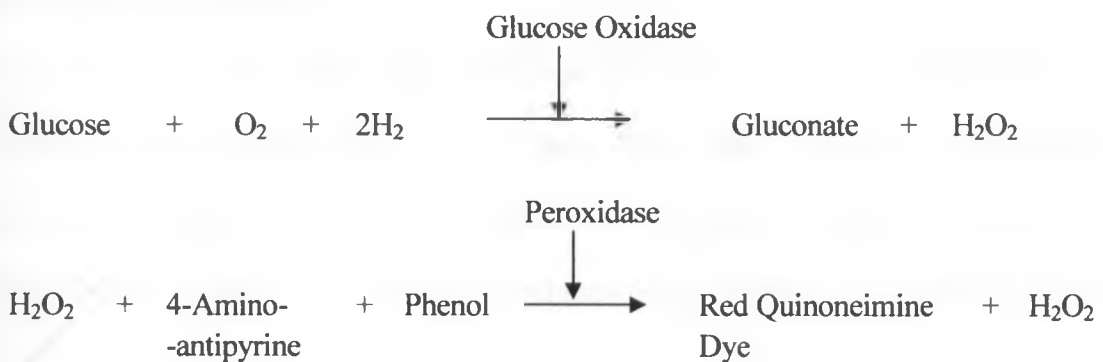
The drug Streptozotocin (STZ) is a glucose analogue (N-[methyl-nitro-carbamoyl]-D-glucosamine) that specifically damages pancreatic β cells (Schein and Loftus, 1968). It is rapidly transported into the β cell via the glucose transporter, Glut 2, and is metabolized by the β cell upon entry (Kroncke *et. al.*, 1995).

STZ acts on the mitochondria by inhibiting the ability to produce ATP and NAD. The major action of STZ on the DNA is believed to be the induction of DNA adducts (Ledoux *et. al.*, 1988). These adducts are in turn repaired by the nuclear excision repair processes. The strand breaks induced during excision repair activate poly (ADP-ribose) polymerase (PARP), which converts NAD into polymers of ADP –ribose at the site of the DNA strand break (Yamamoto *et. al.*, 1981). This leads to NAD depletion. If the DNA repair processes are sufficiently activated, PARP activation can cause the depletion of NAD to non-physiological levels. Normally NAD depletion would be replaced by cellular processes. However, STZ also inhibits the replacement of the NAD by inhibiting mitochondrial ATP generation. Thus, both DNA repair induced NAD depletion and the inability of the cell to replace decreasing NAD concentrations may combine to cause loss of cell viability.

2.10 Measurement of glucose concentration

Available methods for determination of blood glucose concentration fall into two categories, chemical and enzymatic methods. The enzymatic method that was discovered by Muller is based on the enzyme system(s) used and the type of measurements finally employed (Muller, 1928).

In this study, enzymatic method was employed. This procedure has been designed to measure glucose in terms of % absorbance in the calorimeter after enzymatic oxidation. Glucose is oxidized to glucuronic acid and hydrogen peroxide in the presence of glucose oxidase. Hydrogen peroxide further reacts with phenol and 4-aminoantipyrine by the catalytic action of peroxidase to form a red coloured quinoneimine dye complex. Intensity of colour formed is directly proportional to the amount of glucose present in the sample.



If the above method is applied directly to serum or plasma, the problem of inhibitors presents itself. Deproteinization of whole blood is therefore done to remove various interfering substances present in red blood cells. Negative errors are caused by ascorbic acid concentration greater than 50 mg/100 ml, bilirubin, haemolysis and Uric acid (Farnsworth, 1982).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental reagents

All reagents used in this study except solvents used in bulk extraction of crude extracts were of analytical grade. Streptozotocin was purchased from Sigma –Aldrich co, USA. Glucose was assayed using kits from Human Diagnostics[®], Wiesbaden, Germany. Glibenclamide from Roche Diagnostics[®] Germany, was purchased from Pentapharm Chemists Ltd, Kenya.

3.2 Acquisition, Care and Maintenance of experimental Animals

Fourty two male Wistar rats (60 days old, 185 ± 15 g) were used for the study. They were obtained from the Biochemistry Department, University of Nairobi. The animals were housed seven in one polypropylene cage with water available *ad libitum* during different treatments. They were fed on standard mice cubes (Unga[®] Ltd, Nairobi) except during the day of the blood sampling when animals were used after an overnight fast. Steady diabetes was confirmed regularly by measuring and noting the fasting blood glucose level values.

Twenty five adult male rabbits weighing 2-2.5 kg were obtained from Ngong (Kenya) Animal farm. They were housed in individual stainless steel wire mesh tray cages. They were fed on a standard pellet diet (Unga[®] Ltd, Nairobi). The rabbits were given hay and green vegetable material as supplements. Water was provided *ad libitum*.

The animals were held in the animal house of the School of Biological Sciences, University of Nairobi, Chiromo Campus. All animals were randomized into various groups at the onset of study, and held in quarantine for three weeks during which they were conditioned and acclimatized to the institution's facilities.

The animals were maintained in a well-ventilated animal house at temperature of 25 °C on a 12 hour dark-light cycle. Animals described as fasted were deprived of food for at least 16 hours, prior to the experiment, allowing access to water only and were deprived of both food and water during the 5 hour-monitoring period of the experiment to minimize the changes in plasma volume. Principles of Laboratory Animal Care (NIH publication, 1985) were followed.

3.2.1. Induction of diabetes in rats

Forty (40) fasted rats (185 ± 15 g) were intraperitoneally injected with 50 mg/kg of Streptozotocin (Sigma chemical Co. (St. Louis, Mo, USA)), (Kedar and Chakrabarti 1983) in freshly dissolved ice cold 0.1M phosphate buffer pH 4.5, while the control animals were similarly injected only with 1 ml/kg of vehicle by the same route. Forty eight (48) hrs following these injections the blood glucose levels were monitored once in three days, using Glucose oxidase kit (Human, Wiesbaden Germany). Fasted animals with blood glucose levels higher than 200 mg/dL were selected for the study. All other conditions, food intake, water intake were examined everyday after STZ injection.



Figure 1: New Zealand white rabbits that were used for the study. They were each housed in individual steel cages.



Figure 2: Wistar rats that were used for the study. They were housed seven per cage.

3.3 Plant materials

3.3.1 Collection and identification of the plants

Aloe leaves (Figure 3) were obtained from Maralal, (Samburu district), 400 km north of Nairobi, Kenya. Specimens of neem leaves (Figure 4) were collected from Kilifi in Rea Vipingo plantation, 560 km south east of Nairobi.

Specimens of stinging nettle leaves (Figure 5) were collected from Molo area 166 km north west of Nairobi. The nettle leaves were gathered from wild trees to ensure best complement of natural elements. The altitude temperature, rainfall and humidity data of these areas is presented in Appendix 1. These leaves were identified at the University of Nairobi herbarium, School of Biological Sciences and their authentic voucher Specimens deposited in the same herbarium for future reference. Harvesting was done on a dry day. Pesticide-free stinging nettle leaves were collected before flowering. Perfect clean, dust free unblemished leaves free from insect bites or discolourations, were harvested. This was done by use of a pair of secateurs counter intuitively in order to ensure little damage to the plant. The collected neem and nettle leaves were loosely packaged in different brown-paper bags that had previously been labeled (variety, source and the collection date).

3.3.2 Preparation of plant materials

The neem and nettle plant leaves were quickly dried under a shade at a temperature of below 30°C in a dust-damp-free environment that was free of contamination for 6 days to a constant weight. The dry brittle leaves were then pulverized into fine powder in a hammer mill that was fitted with a sieve of 1 mm diameter (Mwangi, 1982). This allows a greater surface area for the release of active compounds.



Figure 3: *Aloe secundiflora* plant showing small bright red tubular flowers and thick, tapered strongly cuticularized leaves growing in a rosette pattern.

(Photograph was taken at Wamba in Samburu district. Scale 1:10)



Figure 4: *Azadirachta indica* tree, fast growing evergreen tree, with a round and dense crown. Inset photo showing white and fragrant flower inflorescences.

(Photograph was taken at Moana field station in Mombasa. Scale: 1:27)

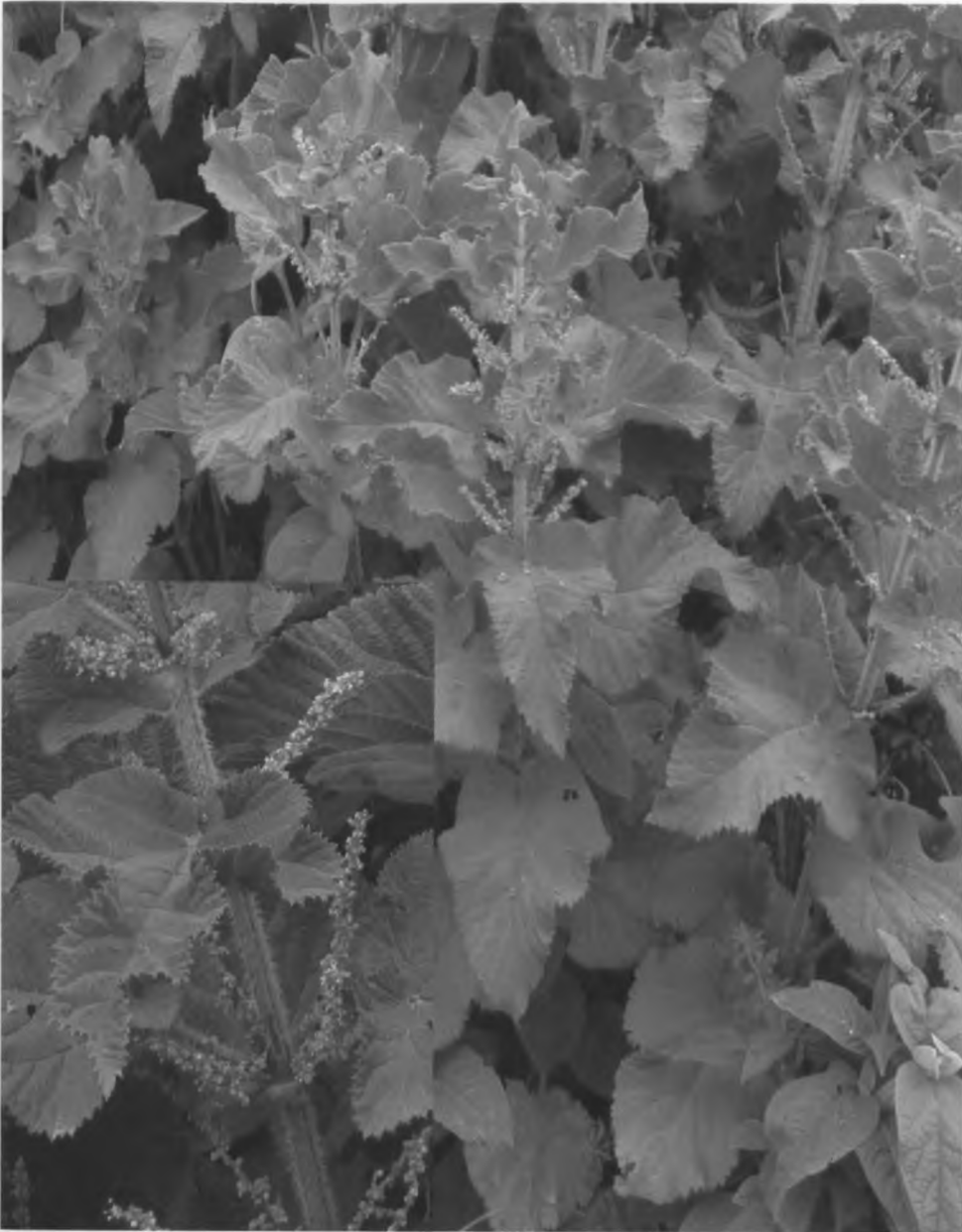


Figure 5: *Urtica dioica* plant showing characteristics four sided stems armed with stinging hairs and tinged pink flower clusters. The plant grows in colonies.

(Photograph was taken at Molo Township in Molo district. Scale 1:12)

3.3.2.1 Extraction of neem leaf

The ethanolic extract of neem leaf was prepared according to the procedure described by Chattopadhyay (1998) (Figure 6). Cold extraction was employed and ethyl alcohol used as a dissolvent. 1kg pulverized *A. indica* leaf powder was extracted using 3L of 60% ethyl alcohol (1:3 w/v). The 1:3 w/v mixture was soaked in a tight opaque container at room temperature for a period of two weeks during which time, the mixture was constantly agitated by use of a wooden rod. After which the supernatant was decanted and filtered using a vacuum pump. Resultant filtrate was concentrated under reduced pressure (temperature 40°C) using a rotavapour and finally dried in a vacuum freeze drier to a constant weight avoiding over drying. The recovered ethyl alcohol was collected, measured and recorded. The extraction procedure was repeated three times to assume total exhaustion of 60% soluble fraction. The residue collected (140.6 g/kg of neem leaf powder) was thick green in colour and gummaceous in nature. The crude extract powder was kept dessicated at 4°-5°C in labeled glass-jars till formulation. Nettle leaf powder was treated in the same way. Residue collected was 110 g/kg of nettle leaf powder.

Similarly 15 samples of 10 g batches of *A. indica* and *U. dioica* leaf powder were each extracted 3 times with 30 ml of 60% ethanol for 14 days. The extracts were decanted and purified following the standard procedure (Figure 6). The yields of small samples were obtained.

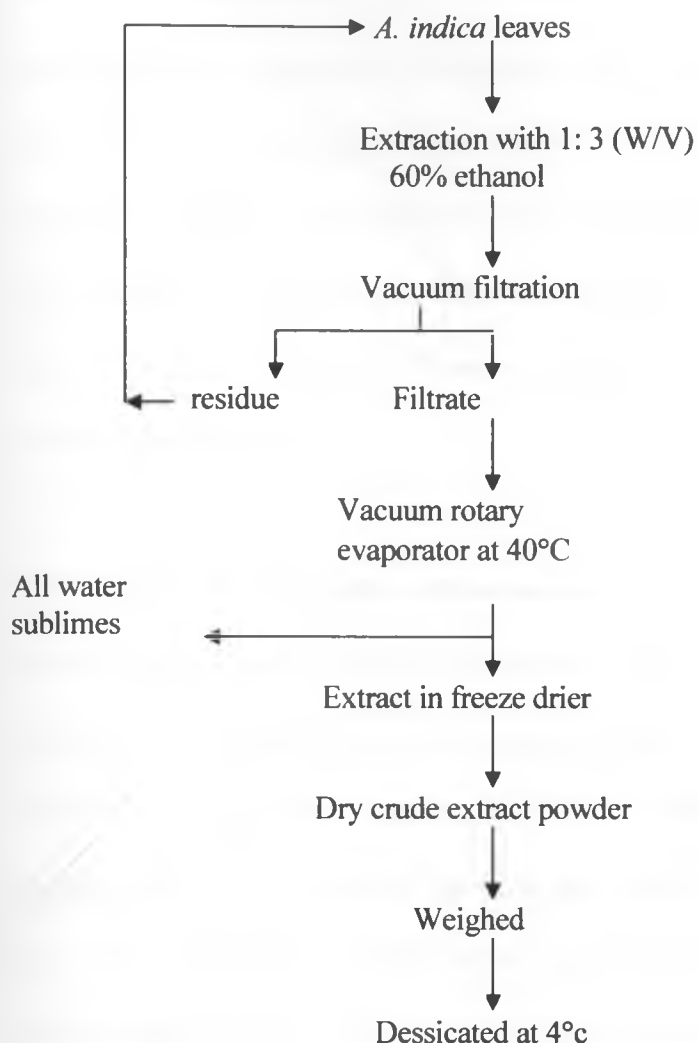


Figure 6: A flow chart showing the procedure for the preparation of dry crude extract of *A. indica* leaves from 60% ethanol (Chattopadhyay, 1998).

3.3.2.2 Preparation of *Aloe secundiflora*

Three fleshy leaves per plant were cut off close to the base of the plant and washed with water. The leaves were immediately placed with the cut end downwards into V-shaped wooden trough to allow the sap juice oozing from the leaves to flow on its sides into a tapping vessel placed beneath. When the vessels receiving the juice filled, the later were removed and the contents weighed. The juice sap was dried in an open copper vessel at a temperature of 80°-90°C to expel the water, concentrate and stabilize it (Figure 7).

The residue collected (0.2 g/kg of aloe leaf crystals) was black with small light green tincture and had a characteristic disagreeable odour, sour, nauseating and a very bitter taste.

This crude extract was kept desiccated at 4°-5°C in labeled glass-jars till formulation.

Similarly 15 samples of 1kg batches of *Aloe secundiflora* leaves were each placed with the cut end downwards into V-shaped wooden troughs to allow the sap juice ooze from the leaves. The juice was dried following the procedure in Figure 7. The yields of small samples were obtained.

3.3.2.2.1 Qualitative analysis on Aloe solution

General tests included: Solubility in alcohol, where 50 ml of alcohol was added to 1 gramme of Aloes and the mixture gently heated and then cooled. Borax Test for Anthranols: 1 ml of this filtrate was diluted with 100 ml of water. Aqueous solution of sodium borate (1 in 20) was added. Modified Borntrager's test for C-glycosides: 1 ml of this mixture was diluted with 100 ml of water and mixed together with 10 ml of benzene and allowed to separate. The benzene layer was shaken with few drops FeCl_3 and dil. HCl. In order to render the medium alkaline, 5 ml of Ammonium hydroxide was added. Observations were recorded.

Specific tests included: 5 g of Aloe crystals were dissolved in 100 ml of double distilled water to form Aloe solution which was subjected to isobarbaloin tests (Trease and Evans, 1978). The Nitric acid test where 2 ml of Nitric acid was added to 5 ml of Aloe crude extract; The Nitrous acid test where half spatula full of sodium nitrite and 1M of acetic acid was added to 5 ml of 2% aqueous solution of Aloe crude extract and the Klunge's isobarbaloin test in which a drop of saturated Copper Sulphate solution followed by 1 g of Sodium Chloride and 10 ml of 90% ethanol was added to 20 ml of aqueous 0.5% solution of aloes crude extract.

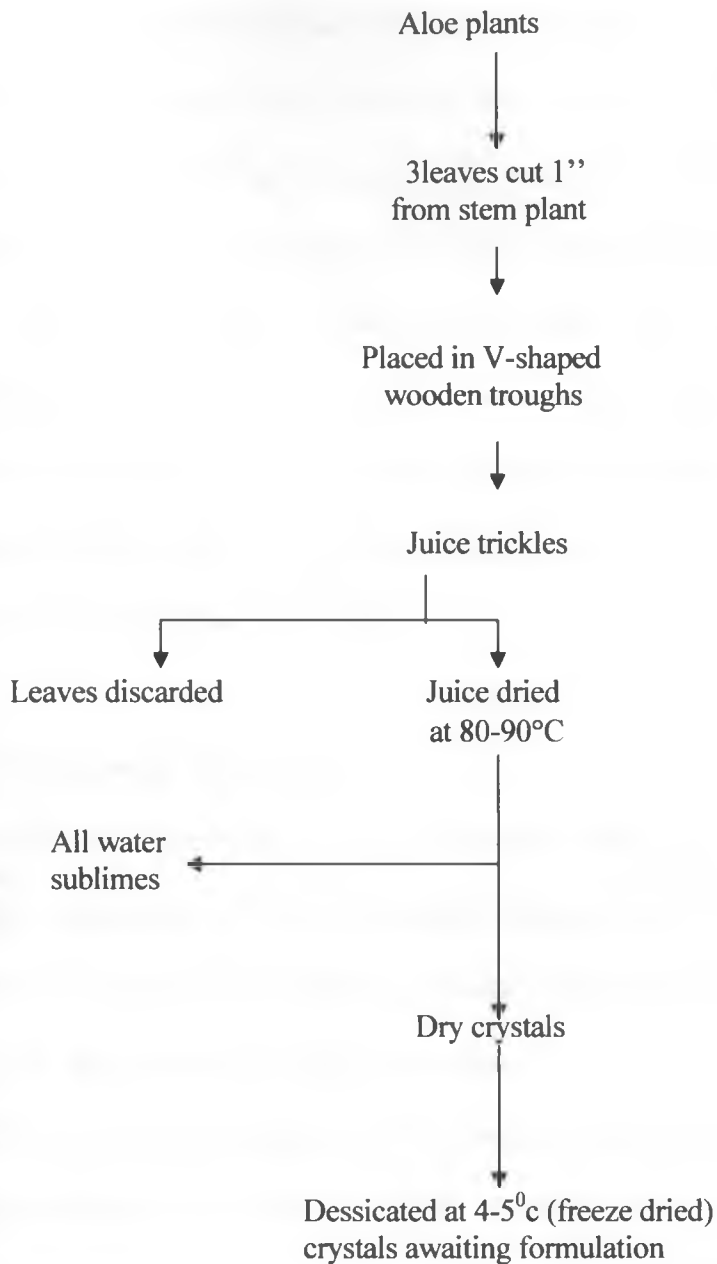


Figure 7: A flow chart showing processes involved in preparation of dried sap of *Aloe secundiflora* leaf extract.

3.3.3 Preparation of extracts for administration

All the additives used in formulation were natural plant products, and the form of drug administration was syrup, which consisted of a concentrated dissolution of sweetener and plant extract.

3.3.3.1 Carboxymethyl-cellulose (CMC) stock solution

Six (6) kilograms (kg) of CMC stock solution was made using 300 grams (g) of CMC powder (5%), 6 g of Sodium Benzoate (0.1%), 6 g of citric acid (0.1%) and distilled water. 5.7 litres of distilled water was warmed to 40°C and emptied into a high speed stirrer glass tank (StHWABEN German), covering the flat rotary blade with the mixture to achieve perfect stirring during mixing. The stirrer was switched on and Citric acid added followed by Sodium Benzoate. The CMC powder was then introduced gradually until the mixture was homogenous, which took 2½ hours. This 5% stock solution CMC gel was used for the subsequent formulations.

3.3.3.2 Preparation of Aloe syrup

To make 3 kg of aloe syrup, 150 g of snow flakes (5%), 60 g of Aloe crystals (2%), 60 g of CMC stock solution (2%), 30 g Sodium Saccharin (1%), 6 g of lemon grass flavour (0.2%), 6 g Amaranth dye (0.2%), 3 g Sodium Benzoate (0.1%), 3 g Citric acid (0.1%) were used. The procedure consisted of two steps:

In the first step, the snowflakes was dissolved in 1 litre of cold distilled water. This was heated gradually over a water bath at 40 °C while agitating with a wooden rod for 10 minutes to a thick homogenous mixture. 1200 g of the CMC stock solution was added to the thickened snowflakes and agitated and mixed with the solution resulting from step two.

In step two, Aloe crystals were mixed with the 600 mls of distilled water maintained at 40 °C in a water bath. The mixture was agitated using a wooden rod until the crystals dissolved. Sodium Saccharin was added into the warm Aloe solution followed by the Citric acid before the addition of Sodium Benzoate and pineapple flavour. This mixture was emptied into the container with solution from step 1, and amaranth extract dye finally added. After this mixing, 1 ml of Aloe syrup contained 50 mg of snowflakes, 20 mg of aloe

dried sap crystals, 20 mg of CMC, 10 mg of Sodium Saccharin, 2 mg of lemon grass flavour, 2 mg of amaranth dye, 1 mg of Citric acid and 1 mg of Sodium Benzoate. Aloe placebo was prepared in the same manner except instead of using 60 g of aloe crystals the syrup was topped up with distilled water.

3.3.3.3 Preparation of nettle syrup

Nettle syrup was prepared in the same manner as 3.3.3.2. To make 3 kg of nettle syrup, 150 g of snow flakes (5%), 60 g of CMC stock solution (2%), 33 g of nettle powder (1.1%), 6 g of lemon grass flavour (0.2%), 6 g Amaranth dye (0.2%), 3 g Sodium Benzoate (0.1%), 3 g Citric acid (0.1%), 3 g Sodium Saccharin (1%) were used.

1 ml of nettle syrup contained 50 mg of snowflakes, 20 mg of CMC, 11 mg of nettle crude extract, 2 mg of amaranth dye, 2 mg of lemon grass flavour, 1 mg of Citric acid, 1 mg of Sodium Benzoate and 1 mg of Sodium Saccharin. Nettle placebo was prepared in the same manner. Instead of using 33 g of nettle powder the syrup was topped up with distilled water.

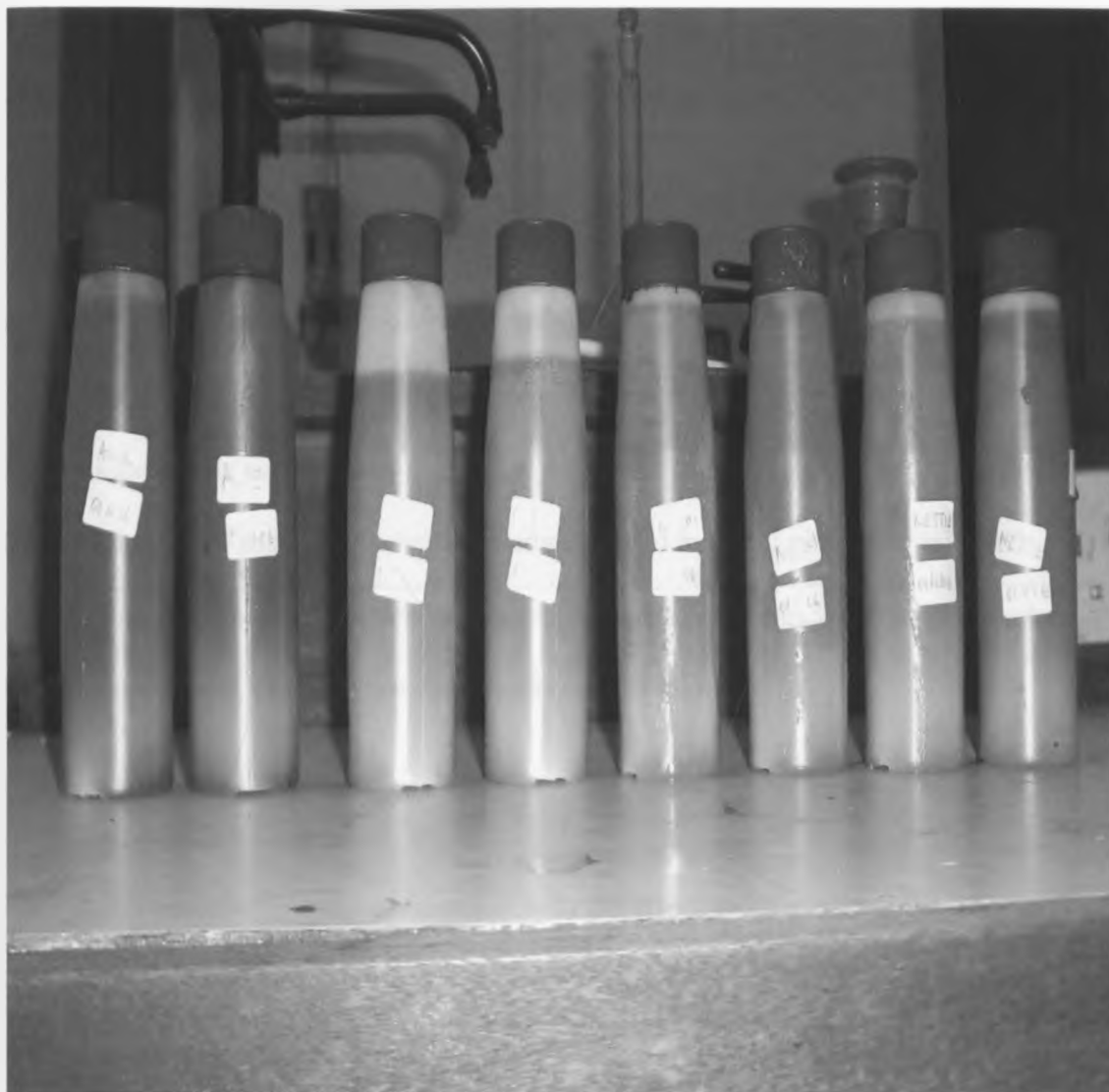


Figure 8: From left: Packed aloe syrup, placebo, neem and nettle syrup ready for administration to the test animals. These samples were stored at 4 °C.

3.3.3.4 Preparation of neem syrup

Neem syrup was prepared as described in section 3.3.6.2. To make 3 kg of neem syrup, 42.18 g of neem (14.1%), 150 g of snowflakes (5%), 60 g of CMC stock solution (2%), 6 g of lemon grass flavour (0.2%) 6 g Amaranth dye (0.2%), 3 g Sodium Benzoate (0.1%), 3 g Citric acid (0.1%), 3 g Sodium Saccharin (1%) was used.

1 ml of neem syrup contained 50 mg of snowflakes, 20 mg of CMC, 14.06 mg of crude neem extract, 2 mg of amaranth dye, 2 mg of lemon grass flavour, 1 mg of Citric acid, 1 mg of Sodium Benzoate and 1 mg of Sodium Saccharin. Neem placebo was prepared in the same manner. Instead of using 42.18 g of neem crude extract, distilled water was used to top up the syrup. The pH of the prepared samples was taken before storage at 4 °C.

3.4 Glibenclamide suspensions

Five Milligrams (5 mg) Glibenclamide was suspended in 21 ml phosphate buffered saline (PBS). 4 ml propylene glycol was added and the mixture kept in an ultra sonic water bath for 45 minutes until a homogenous suspension was obtained.

3.5 Administration

Each group of animals was administered with the effective dose once a day. The extracts were administered in their syrup form. A mouth gag and a small diaphragm pediatric feeding tube were used for intra-gastric oral intubation (Hunt and Harrington, 1974) in the rabbits. An intra-gastric 15G (11 cm long) gavage was used for administration to the rats.

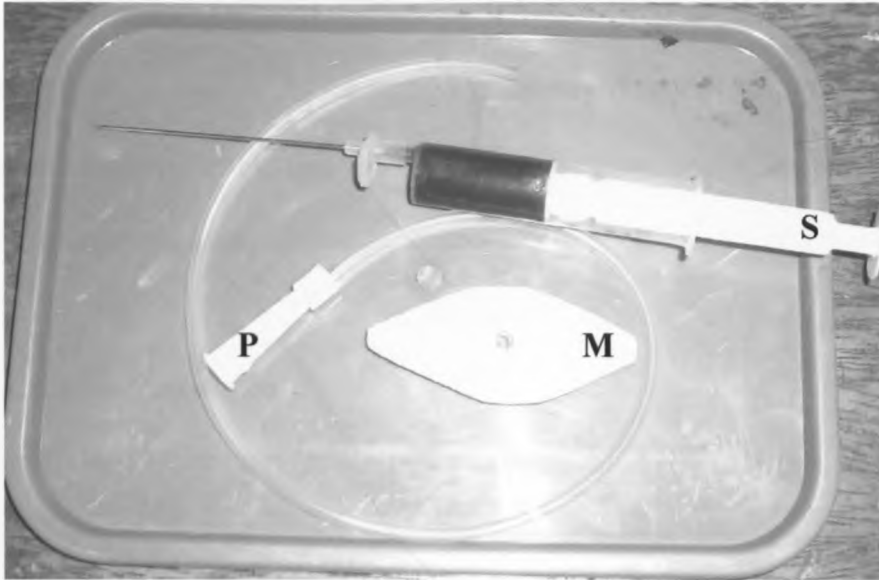


Figure 9: Wooden mouth gag (M), paediatric tube (P), and a syringe (S) (with Aloe syrup) used in extract administration in the rabbits.

3.6 Glucose determination procedure

(Barham and Trinder, 1972).

Plasma was analyzed for glucose content using a glucose oxidase kit. Blood samples were collected from the marginal ear vein for the rabbit and tail vein in the rat by use of a heparinized capillary tube. At every instant 50 μ l of blood was collected and placed in 500 μ l of deproteinizing solution (uranyl acetate). The solution was centrifuged for 5 mins at 3000 rpm (Beckman, Microfuge 18, Germany). 1000 μ l of enzyme reagent was added to 50 μ l of the supernatant.

A blank of 50 μ l-distilled water in 1000 μ l of the enzyme reagent was also prepared for calibration. A 50 μ l standard containing 1000 μ l of the enzyme reagent was ran alongside. The contents were incubated at room temperature for 5 minutes. The absorbance was

measured against the reagent blank within 60 mins by use of a spectrophotometer (photo electric instruments PVT Ltd, Judhpur) at 520 nm.

Intensity of the red dye colour formed is directly proportional to the amount of glucose present in the sample. For quantitative results the absorbance of the sample and the standard were measured and read against the blank.

3.7 Experimental design

Twenty five (25) normoglycaemic rabbits were divided into 5 groups of 5 rabbits each. Group A consisted of control normoglycaemic rabbits treated with placebo; Group B normoglycaemic rabbits treated with aloe extract; Group C normoglycaemic rabbits treated with neem extract; Group D normoglycaemic rabbits treated with nettle extract and Group E normoglycaemic rabbits treated with glibenclamide.

Thirty five (35) diabetic and 7 normal rats were divided into 6 groups of 7 rats each. Group 1 consisted of Non-diabetic normal control rats; Group 2 diabetic control rats treated with placebo; Group 3 diabetic control rats treated with aloe extract; Group 4 diabetic rats treated with neem extract; Group 5 diabetic rats treated with nettle extract and Group 6 diabetic rats treated with glibenclamide.

3.7.1 Experimental procedures

3.7.1.1 Assessment of acute effect of different doses of ethanol extracts of *A. secundiflora*, *A. indica* and *U. dioica* on fasting blood glucose level in normoglycaemic rabbits.

Five (5) groups each consisting of five overnight fasted normoglycaemic rabbits were treated as follows: Three (3) groups were orally administered 10 mg/kg each of *A. secundiflora*, *A. indica* and *U. dioica* extract. The fourth and fifth sets were administered 5

mg of glibenclamide, and placebo respectively. The effects of the extracts were monitored over a period of four weeks against normal rabbits fed on placebo. The blood glucose level was determined in 2 phases:

- i) Blood glucose estimated just before extract administration on day of bleeding.
- ii) Blood glucose estimated for duration of three (3) hours after extract administration.

This was repeated for the extract concentrations of 20, 40, 80 and 100 mg /kg body weight.

3.7.1.2 Determination of chronic effect of graded doses of different ethanol extracts of *A. secundiflora*, *A. indica* and *U. dioica* in normoglycaemic rabbits.

Five (5) groups each consisting of five overnight fasted normoglycaemic rabbits were treated as follows: Three (3) groups were orally administered 10 mg/kg each of *A. secundiflora*, *A. indica* and *U. dioica* extract. The forth and fifth sets were administered 5 mg of glibenclamide, and placebo respectively. The extracts were administered daily for a period of four weeks and their effects monitored. The blood glucose level was estimated just before extract administration on day of bleeding. This was repeated for the extract concentrations of 20, 40, 80 and 100 mg /kg body weight.

3.7.1.3 Screening study of dosages and Glucose tolerance test

In the screening study, 20 Wistar rats weighing 100 ± 10 g were used. They were divided into 4 groups of five each. The hypoglycaemic effect of the extracts was tested by oral administration to the rats using a concentration of 20 mg/kg, 40 mg/kg, 80 mg/kg and 100 mg/kg b.w. An oral glucose tolerance test was used to continue studying the concentrations of the three extracts that had shown hypoglycaemic effect in the screening tests. The 20 Wistar rats were divided into 4 groups of five each. The rats were fasted overnight. 210 min before the oral administration of glucose (3 g/kg b.w.), three (3) groups of the rats were orally given 80 mg/kg each of *A. secundiflora*, *A. indica* and *U. dioica* extracts. The forth

group (control) was given 0.9% NaCl. Blood glucose concentrations were measured in blood taken after small tail vein incision at -210, 0, 30, 60 and 120 minutes.

3.7.1.4 Acute effect of the effective dose of ethanol extracts of *A. secundiflora*, *A. indica* and *U. dioica* on blood glucose level in fasting diabetic rats.

Six (6) groups of overnight fasted rats were treated as follows; effective doses of *A. secundiflora*, *A. indica* and *U. dioica* extracts were orally administered to 3 groups of diabetic rats every day for six weeks. The fourth group of rats was fed on 5 mg of glibenclamide, a standard drug. The fifth group was fed on placebo. The effects of the extracts were monitored over a period of six weeks against normal rats fed on placebo. The blood glucose level determined in 2 phases:

- i) Blood glucose estimated just before extract administration on day of bleeding.
- ii) Blood glucose estimated for a duration of three (3) hours after extract administration

3.7.1.5 Assessment of the chronic effect of effective dose of ethanol extracts of *A. secundiflora*, *A. indica* and *U. dioica* in diabetic rats.

Six (6) groups of overnight fasted rats were treated as follows; effective doses of *A. secundiflora*, *A. indica* and *U. dioica* extracts were orally administered to 3 groups of diabetic rats every day for six weeks. The fourth and the fifth group were each fed on 5 mg of glibenclamide (a standard drug) and placebo respectively. The effects of the extracts were monitored over a period of six weeks against normal rats fed on placebo. The blood glucose level was estimated just before extract administration on day of bleeding. All rats from the six groups were weighed on a weekly basis using beam balance scale. Mean weights from each replication were recorded to establish the weight gain (growth rate) against the control normal rats.

3.7.1.6 Urine output in normal rats fed on nettle extract

Urine output of two groups of normal Wistar rats fed on ethanol extract of *U. dioica* and placebo was measured by collecting urine produced in 24 hrs by each group of rats. The initial number of rats per group in these experiments was 5 and the tests were conducted in triplicate. Each group was kept in a modified metabolism cage for 24 hrs where urine and faeces were collected separately. Several drops of toluene were added to the urine collection beaker to inhibit microbial growth (Alderson *et. al.*, 2004). Water consumption was measured by weighing the supplied drinking water before and after 24hrs.

3.9.1.7 Assessment of the effective dose of *A. secundiflora* leaf extract on BGL in human clinical trials

Written informed consent was obtained from the patients after approval of the investigation protocol by our institutional Ethical committee. In an open uncontrolled study in an Alternative Medicine Center located in Nairobi, Kenya, fifteen patients, six males and nine females aged between 27-82 years were enrolled for the study. They were administered the formulated aloe extracts at a dose of 80 mg/kg and their blood glucose level was monitored until it stabilized. The subjects were not on any other medication. Assessment of fasting blood glucose level was monitored by use of a glucometer (Elite[®], Bayer, Arkansas, U.S) and this was done throughout the visit and compared with the baseline value.

3.7.1.8 Effect of extracts on glucose absorption through a rabbit gut preparation

Three overnight fasted rabbits were sacrificed under anaesthesia and small jejunum segments (each about 6 cm) removed from the abdomen. They were rinsed with KRB by pushing the solution gently from the syringe. Four sets (in fives) of the segments were placed in well-oxygenated KRB containing 11.1mM glucose along with 40 mg concentrations of the plant extracts of *A. secundiflora*, *A. indica*, *U. dioica* and buffer as

control. The fifth set was placed in 100 µg of acarbose. These were incubated at 37 °C for 2 hr in CO₂ incubator under 95 % O₂ and 5 % CO₂ atmosphere. Aliquots of 25 µl were removed from the incubation mixture at 0, 30, 60, 90 and 120 minutes and change in the glucose concentration in the medium was determined.

3.7.1.9 Histopathology of the kidneys and pancreas

After 6 weeks of treatment the six groups of Stz-induced diabetic rats were sacrificed by ether anaesthesia. Kidneys were removed and weighed. To determine wet kidney weight, the kidneys were decapsulated and placed on tissue paper for 1 minute, then transferred to a scale and weighed. The Pancreatic samples were fixed in Bouin's solution, dehydrated in gradual alcohol (80-100 %) by use of a tissue processor (Shandon Elliot, Scientific Company Ltd., London, UK.), cleared in xylene and subsequently embedded in oven warmed paraffin. Sectioning of the samples was done using a microtome (Leitz Wetzlar, Germany) the cutting knife (Leitz, Germany) was set at an angle of 40° as recommended by manufacturers; the thickness of the sections set at 5µm after which the cutting proceeded. The ribbon sections were picked from the knife using a brush and placed in water kept at 37 °C using a water bath (Electrothermal® England) during which the slides were labelled with the date of sectioning, type of the tissue and the animal number. A diamond pencil was used to mark around the sections on the slides. The sections were picked onto the slides and warmed on a hotplate (Photax, England.). The sections were dewaxed and rehydrated. Histologic examination and observations were carried out on hematoxylin –eosin dye stained sections.

3.8 Photography

Photographs were taken using a digital camera.

3.9 Data collection and presentation

In all the experiments food and water intake were determined every other day while the weight was done weekly during the experimental time. Blood glucose level determination was done at 6-day interval for the rabbits and 4-day interval for the diabetic rats. Extracts were administered daily for 4 weeks for the normoglycaemic rabbits and 6 weeks for the diabetic rats. Data on BGL was presented as mean \pm mg /dL. Data on weight was presented as g / rats / day. Data on water intake presented as ml /rat / day.

3.10 Data analysis and graphics

Results are expressed as means \pm S.E.M. or \pm SD. Unpaired sample t-test was employed to assess the change in blood glucose for each group at each time point and to identify the differences between the treatment groups. Paired sample t-test was used to compare the value of FBGL, weight, feed and water intake before and after treatment. Relationships between variables were assessed by using Pearson correlation tests. Within group comparisons were performed by analysis of variance ANOVA (Analysis of Variance) test for repeated measurements using SPSS soft ware. Following a significant F value, a post hoc students Turkey's test was performed. Statistical significance was realized at $P < 0.05$ or less, in order to approve the null hypothesis for individual parameters. SPSS (Statistical Programme for social sciences) 11.0 Software package for Windows TM was used in data analyses and construction of figures. Microsoft office word TM 2003 programme was also used in graph presentations.

CHAPTER FOUR

RESULTS

4.1 Qualitative analysis on Aloe solution and the yield obtained from dry leaves of *A. indica* and *U. dioica* with 60% ethanol concentration

The mean yield of 15 samples of dry leaf powder from 10 g batches of *A. indica* extracted 3 times with 30 ml of 60 % ethyl alcohol following the standard procedure (Figure 6) was 18.80 ± 0.200 (Mean \pm SE). The mean yield of 15 samples of dry leaf powder from 10 g batches of *U. dioica* extracted 3 times with 30 ml of 60% ethyl alcohol following the standard procedure (Figure 6) was 12.27 ± 0.080 (Mean \pm SE). 15 samples of 1kg of Aloe leaves each yielded a mean of 20 ml of sap juice which produced a mean yield of $0.021 \pm 0.0012\%$ (Mean \pm SE). All qualitative analysis by use of the Nitric acid, Nitrous acid and Klunge's isobarbaloin tests confirmed presence of isobarbaloin in the Aloe solution (Table 1).

Table 1. Qualitative analysis on Aloe solution showing the colour reactions of the Aloe juice powder

Chemical test	Colour change	Deductions / Possible component	Comments
Solubility in alcohol	Near clear solution obtained	Almost entirely soluble in alcohol	Solution not very clear may be because of gum and inorganic impurities.
Borax test for Anthranols	Yielded a green fluorescence	Indicates presence of Aloe	Green coloured fluorescence is due to the formation of aloe emodin
Modified Borntrager's test	A permanent cherry red colour was produced in the lower benzene layer	This indicates presence of C-glycosides	Aloin (or barbaloin) belongs to the class of C-glycoside which does not undergo hydrolysis by heating with dilute acid or alkali. Though it is decomposed with ferric chloride due to oxidative hydrolysis. Hence, the modified Borntrager's test that employs use of FeCl ₃ and HCl.
Nitric acid test	Pale brownish yellow	Indicates presence of <i>Aloe secundiflora</i>	This test is used to differentiate between the different types of Aloes
Nitrous acid test	A deep brown colour changing to wine red colour slowly.	Indicates presence of <i>Aloe secundiflora</i>	This test is used to differentiate between the different types of Aloes
Klunge's test	Faint brown colouration changing to yellow quickly.	Indicates presence of <i>Aloe secundiflora</i>	This test produces different shades of colours depending on the variety of aloes used

4.2 Acute effect of different doses of 60% ethanol extract of *A. secundiflora*, *A. Indica* and *U. dioica* on fasting BGL of normoglycaemic rabbits after 18 hour fasting

Investigations into the effect of ethanol leaf extracts of *A. indica*, *U. dioica* and *A. secundiflora*, on fasting blood glucose level of normoglycaemic rabbits carried out in this study showed that these extracts produced a profound and dose dependent hypoglycaemia. In all the three extracts, increase in extract concentration from 10- 100 mg/kg/day lowered fasting blood glucose levels. This was supported by ANOVA results which showed p-values of $p=0.001$ for 80-100 mg/kg/day concentration, indicating significant differences in means of fasting blood glucose levels across concentrations of the three extracts (Table 2).

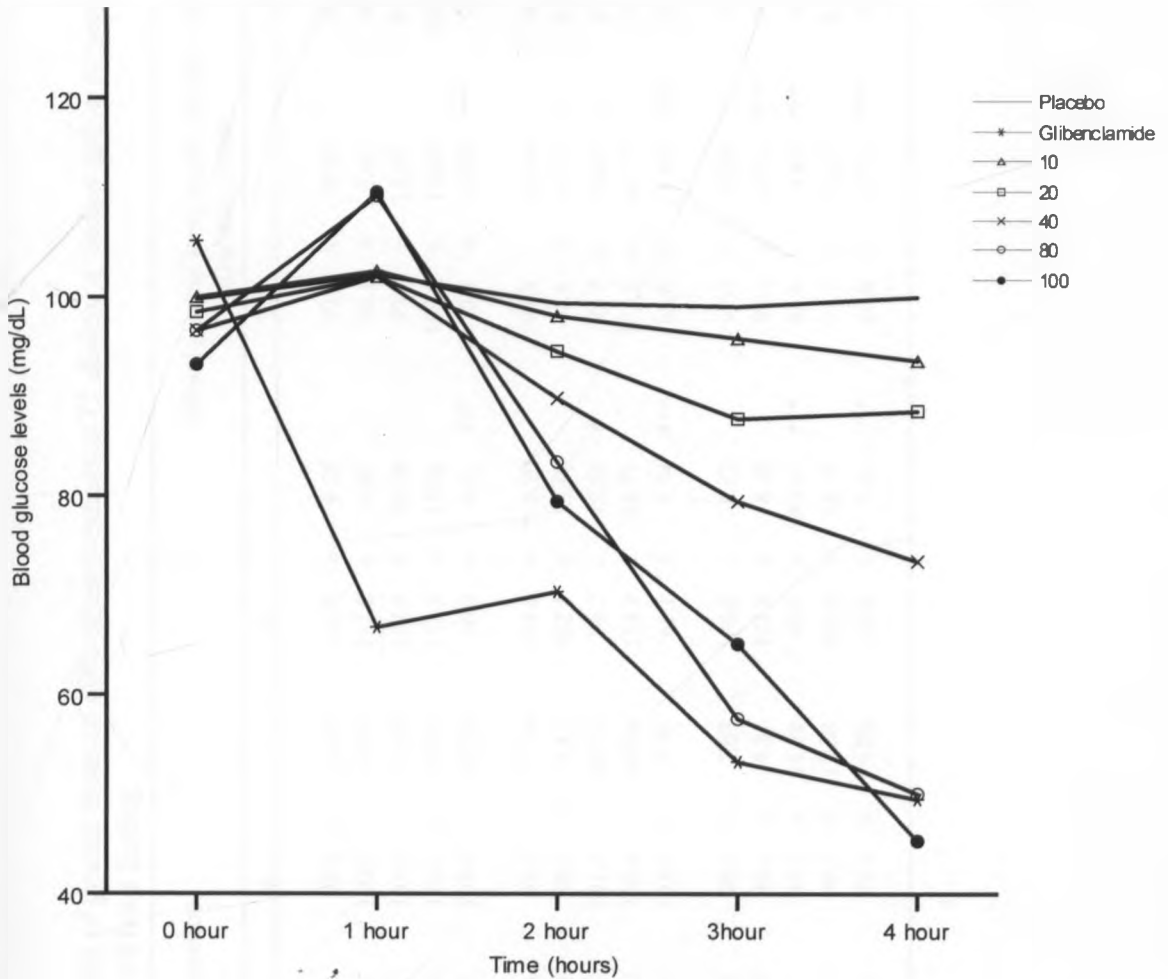


Figure 10: Acute effect of different doses of dried leaf sap of *A. secundiflora* on blood glucose levels of normoglycaemic rabbits.

Table 2: Effect of graded doses of 60% ethanol extract of *U. dioica*, *A. indica* and *A. secundiflora* on fasting blood glucose levels of normal rabbits after 18 hour fasting.

Extract concentration	Blood glucose level mg/dL (mean \pm SD)									
	0		1		2		3		4	
10	A	99.8 \pm 8.64	102.4 \pm 9.25	99.5 \pm 9.99	99.1 \pm 8.41	100.0 \pm 9.81				
	B	100.0 \pm 4.76	102.8 \pm 4.82	98.1 \pm 3.61	95.8 \pm 3.25	93.7 \pm 4.85	**			
	C	103.1 \pm 14.63	102.4 \pm 19.06	99.3 \pm 13.55	97.7 \pm 12.6	96.5 \pm 12.97				
	D	110.5 \pm 15.80	113.3 \pm 15.68	108.0 \pm 15.82	104.5 \pm 15.6	103.1 \pm 15.41				
	E	105.6 \pm 8.32	66.8 \pm 4.21	***	70.3 \pm 4.09	***	53.3 \pm 3.29	***	49.5 \pm 2.84	***
20	A	99.5 \pm 7.34	101.6 \pm 6.10	98.3 \pm 8.18	98.8 \pm 7.30	100.7 \pm 8.96				
	B	98.6 \pm 5.82	102.1 \pm 5.88	94.6 \pm 5.77	*	87.8 \pm 5.48	***	88.6 \pm 4.31	***	
	C	101.2 \pm 14.93	96.7 \pm 12.56	*	93.7 \pm 12.64	*	91.1 \pm 11.4	**	89.7 \pm 11.78	***
	D	108.4 \pm 14.60	114.3 \pm 14.71	103.8 \pm 13.13	97.7 \pm 12.1	95.2 \pm 12.16	*			
	E	103.1 \pm 7.91	65.1 \pm 4.54	***	68.6 \pm 3.94	***	53.9 \pm 6.50	***	48.8 \pm 3.02	***
40	A	98.8 \pm 7.69	100.6 \pm 6.62	97.7 \pm 8.38	97.8 \pm 7.83	100.1 \pm 9.08				
	B	96.6 \pm 4.51	102.2 \pm 8.18	89.9 \pm 6.33	***	79.5 \pm 5.56	***	73.4 \pm 5.06	***	
	C	98.5 \pm 14.28	89.3 \pm 10.96	***	85.4 \pm 9.85	***	78.7 \pm 12.01	***	73.9 \pm 11.93	***
	D	99.7 \pm 12.58	105.0 \pm 12.74	91.7 \pm 12.21	83.0 \pm 8.93	77.3 \pm 7.20	***			
	E	99.1 \pm 6.84	63.3 \pm 5.26	***	66.4 \pm 3.75	***	52.4 \pm 6.18	***	46.6 \pm 2.74	***

Table 2 contd.: Effect of graded doses of 60% ethanol extract of *U. dioica*, *A. indica* and *A. secundiflora* on fasting blood glucose levels of normal rabbits after 18 hour fasting.

Extract concentration	Blood glucose level mg/dL (mean \pm SD)													
	0		1		2		3		4					
80														
A	96.7	\pm 7.62	99.1	\pm 6.67	95.6	\pm 8.44	96.0	\pm 7.40	98.4	\pm 8.66				
B	96.6	\pm 6.64	110.2	\pm 16.18	***	83.5	\pm 5.01	***	57.6	\pm 5.72	***	50.0	\pm 2.39	***
C	95.2	\pm 15.04	75.2	\pm 20.75	***	67.4	\pm 16.37	***	62.2	\pm 10.46	***	52.4	\pm 14.82	***
D	94.5	\pm 10.13	119.6	\pm 10.55	***	80.3	\pm 7.76	***	57.6	\pm 4.81	***	51.3	\pm 6.53	***
E	99.4	\pm 6.43	63.7	\pm 4.81	***	66.5	\pm 3.52	***	50.0	\pm 3.84	***	46.4	\pm 3.14	***
100														
A	99.3	\pm 7.98	101.4	\pm 6.39		98.0	\pm 8.86		98.5	\pm 7.45		100.5	\pm 9.63	
B	93.3	\pm 6.43	110.6	\pm 14.46	**	79.5	\pm 4.54	***	65.1	\pm 4.03	***	45.3	\pm 1.78	***
C	89.8	\pm 14.83	69.6	\pm 10.55	***	63.0	\pm 10.36	***	57.5	\pm 12.73	***	47.03	\pm 10.96	***
D	89.7	\pm 9.47	114.8	\pm 10.58	***	75.5	\pm 8.06	***	53.8	\pm 5.01	***	46.6	\pm 5.59	***
E	97.2	\pm 6.9	62.6	\pm 5.10	***	63.3	\pm 6.19	***	48.8	\pm 3.99	***	45.5	\pm 3.08	***

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ when compared with normal control. Time 0 hours: Initial glucose level before any treatment. $n=5$ in all groups. Five groups consisting of normoglycaemic rabbits were each administered with plant extracts *A. secundiflora* (Group B), *A. indica* (Group C) and *U. dioica* (Group D). The fourth group was fed on placebo as control (Group A), while the fifth set was administered 5 mg glibenclamide (positive control, Group E). Change in the blood glucose levels was studied for four hours.

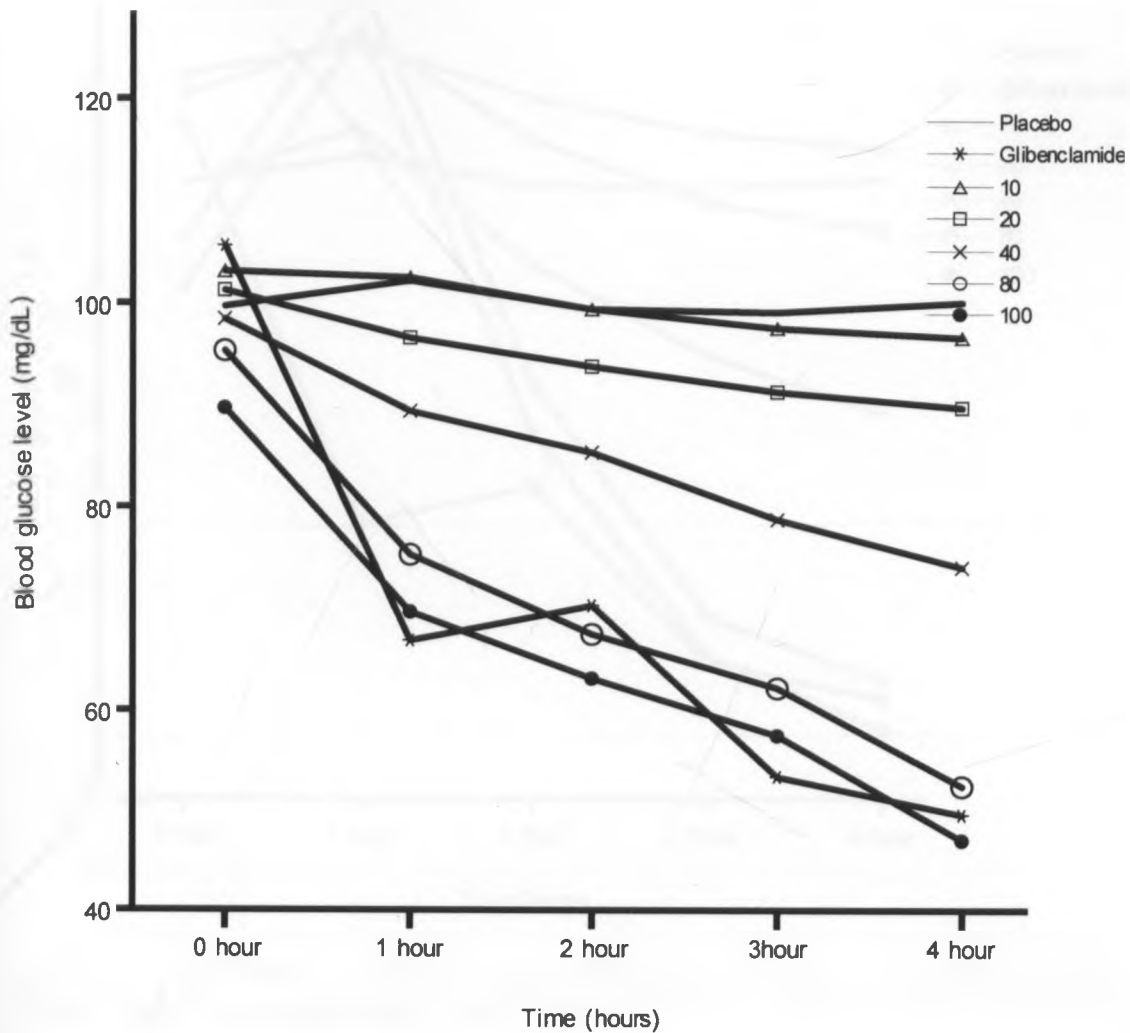


Figure 11: Acute effect of different doses of ethanol leaf extract of *A. indica* on blood glucose levels of normoglycaemic rabbits.

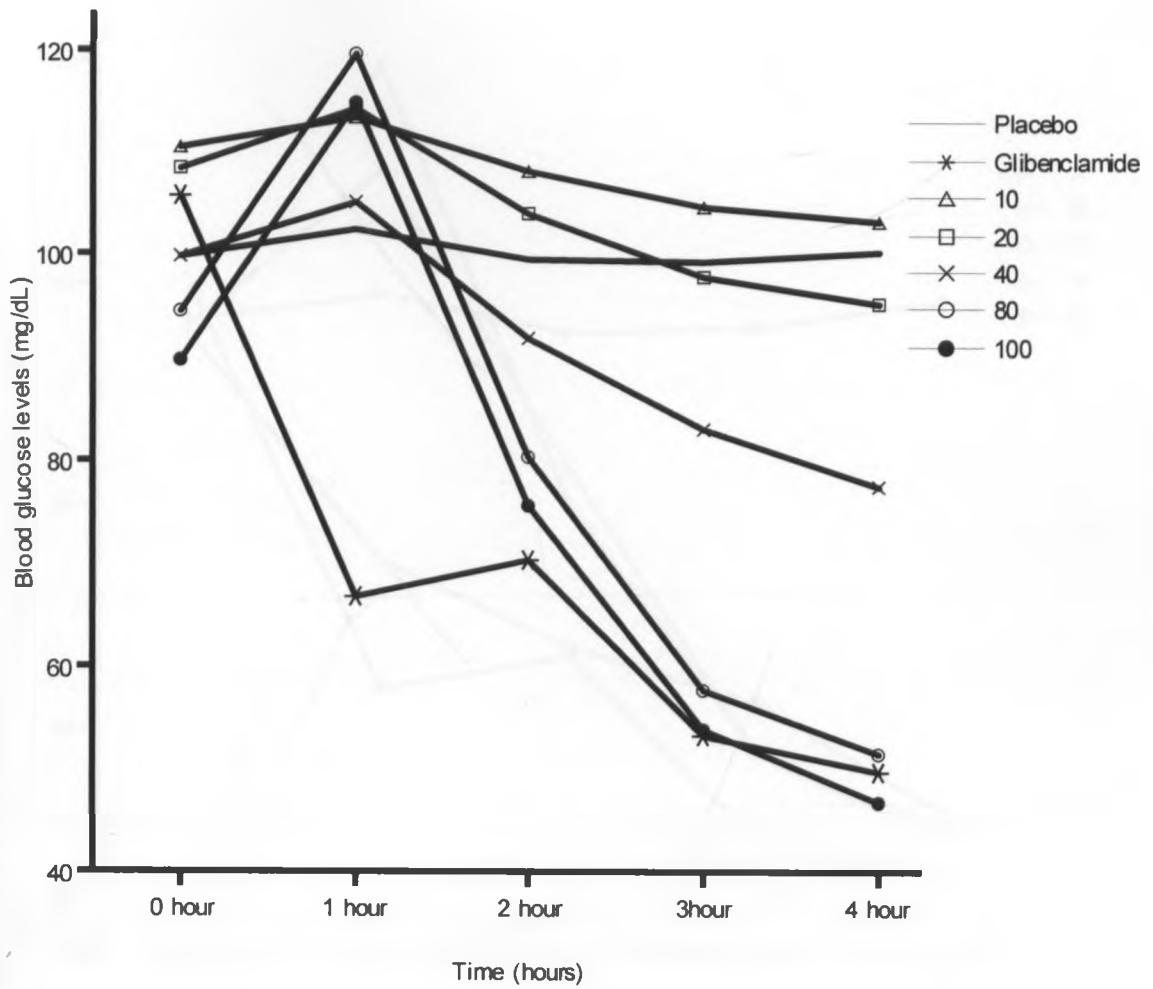


Figure 12: Acute effect of different doses of ethanol leaf extract of *U. dioica* on blood glucose levels of normoglycaemic rabbits.

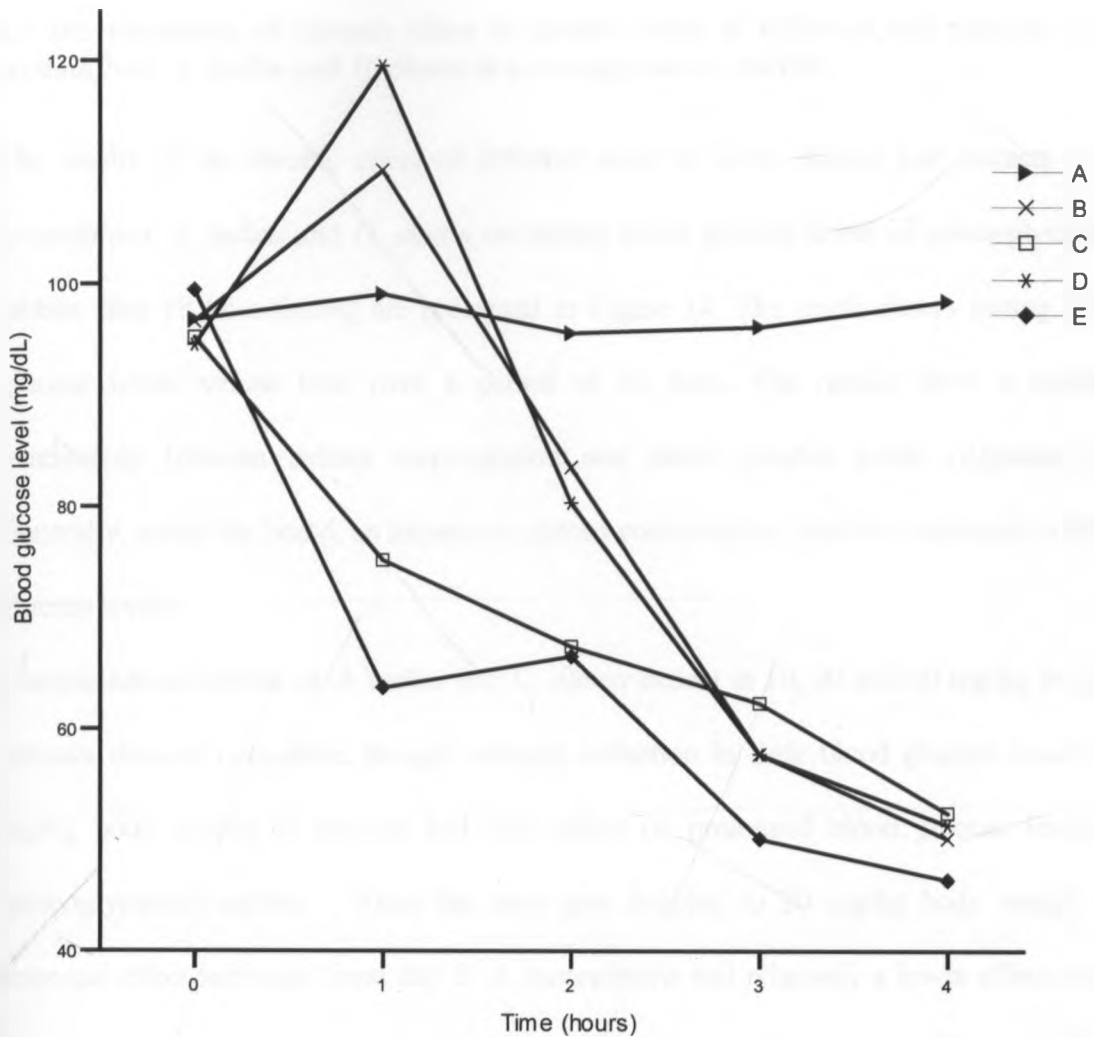


Figure 13: Effect of effective doses of *A. secundiflora*, *A. indica* and *U. dioica* leaf extracts on blood glucose levels of normoglycaemic rabbits. Time 0 hours: Initial glucose level before any treatment. Five groups consisting of normoglycaemic rabbits were each administered with plant extracts *A. secundiflora* (Group B), *A. indica* (Group C) and *U. dioica* (Group D). The fourth group was fed on placebo as control (Group A), while the fifth set was administered 5 mg glibenclamide (positive control, Group E). Change in fasting blood glucose levels was studied for four hours. n=5 in all groups.

4.3 Determination of chronic effect of graded doses of different leaf extracts of *A. secundiflora*, *A. indica* and *U. dioica* in normoglycaemic rabbits

The results of the chronic effect of different doses of 60% ethanol leaf extracts of *A. secundiflora*, *A. indica* and *U. dioica* on fasting blood glucose levels of normoglycaemic rabbits after 18 hour fasting are presented in Figure 14. The graph shows fasting blood glucose levels versus time over a period of 30 days. The results show a negative relationship between extract concentration and blood glucose levels (Appendix 8). Generally, across the board, an increase in extract concentration leads to a decrease in blood glucose levels.

Chronic administration of *A. indica* and *U. dioica* extract at 10, 20 and 40 mg/kg in these animals showed noticeable, though minimal reduction in their blood glucose levels. 10 mg/kg body weight of extracts had little effect on prolonged blood glucose levels in normoglycaemic rabbits. When the dose was doubled to 20 mg/kg body weight the response effect increased from day 5. *A. secundiflora* had relatively a lower effect at this dose. At 100 mg/kg *A. indica* and *U. dioica* extracts had statistically significant effect, $p < 0.05$ against the control (Appendix 8). Animals fed on 100 mg/kg b.w. of *A. secundiflora* had decreased appetite and experienced loose stool.

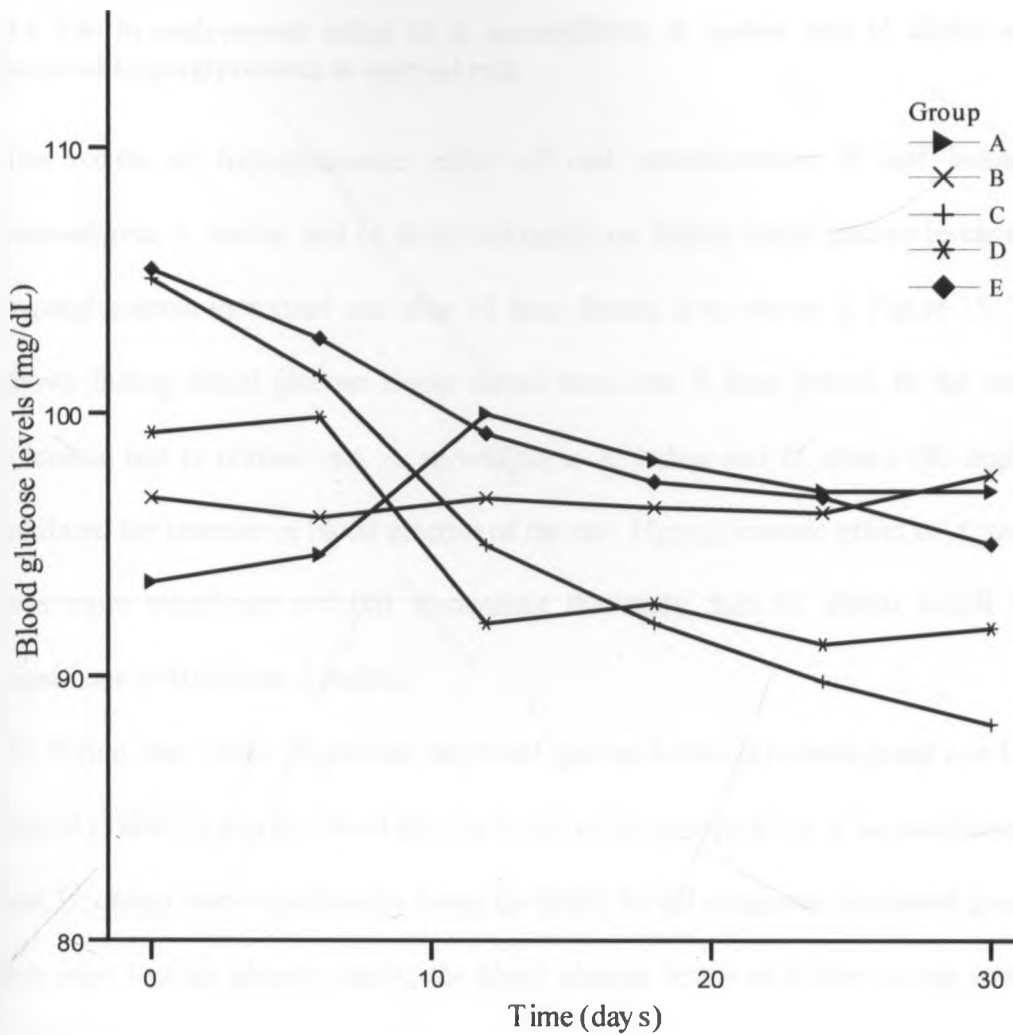


Figure 14: Chronic effect of oral administration of effective doses (80 mg/kg) of *A. secundiflora*, *A. indica* and *U. dioica* on blood glucose level of normoglycaemic rabbits. Time 0 days: Initial glucose level before any treatment. Five groups consisting of normoglycaemic rabbits were each administered with plant extracts *A. secundiflora* (Group B), *A. indica* (Group C) and *U. dioica* (Group D). The fourth group was fed on placebo as control (Group A), while the fifth set was administered 5 mg glibenclamide (positive control, Group E). Change in the blood glucose levels was studied for four weeks. n=5 in all groups.

4.4 The hypoglycaemic effect of *A. secundiflora*, *A. indica*, and *U. dioica* on glucose induced hyperglycaemia in normal rats

The results of hypoglycaemic effect of oral administration of leaf extracts of *A. secundiflora*, *A. indica*, and *U. dioica* 80 mg/kg on fasting blood glucose levels of induced hyperglycaemia in normal rats after 18 hour fasting is as shown in Figure 15. The graph shows fasting blood glucose levels versus time over 5 hour period. In the oral glucose tolerance test in normal rats, *A. secundiflora*, *A. indica* and *U. dioica* (80 mg/kg orally) inhibited the increase in blood glucose of the rats. Hypoglycaemic effect of *A. secundiflora* was more significant $p < 0.001$ throughout the study than *U. dioica* which was more significant $p < 0.05$ than *A. indica*.

At 30 min after intake of glucose, the blood glucose levels of control group was 197.8 ± 2.17 mg/ml (Table 3), and the blood glucose levels of the groups given *A. secundiflora*, *A. indica* and *U. dioica* were significantly lower ($p < 0.001$ for all compared to control group). At 60 min after loading glucose orally, the blood glucose levels of control group was still high (189.0 ± 3.08 mg/ml) whilst the other groups given *A. secundiflora*, *A. indica* and *U. dioica* were significantly lower ($p < 0.001$ for all compared to control group).

Notably, upon oral administration of glucose (3 g/kg b.w.), the fasting blood glucose levels of hyperglycaemic normal rats fed on *A. secundiflora* was markedly lowered at first hour and further returned to the normal range at the second hour while the blood glucose levels of rats fed on *U. dioica* took two hours to return to the normal range. Even after two hours, blood glucose levels of rats fed on *A. indica* had not returned to normal and this was also true for the control rats which remained relatively high at the second hour. This results show that 60% ethanol extract of *A. indica*, *U. dioica* and *A. secundiflora* (at 80 mg/kg) given orally significantly ($p < 0.001$) improved glucose tolerance in glucose-induced normal rats.

Table 3: Hypoglycaemic effect of ethanol extract of *U. dioica*, *A. indica* and *A. secundiflora* on glucose induced hyperglycaemia in normal rats.

Treatment	Blood glucose level mg/dL (mean \pm SD)				
	Duration in mins				
	210	0	30	60	120
1	85.8 \pm 6.06	83.2 \pm 5.26	197.8 \pm 2.17	189.0 \pm 3.08	113.4 \pm 9.99
2	87.0 \pm 4.18	75.6 \pm 3.58	140.2 \pm 3.83 ***	86.4 \pm 3.21 ***	72.4 \pm 1.67 ***
3	90.8 \pm 3.96	87.2 \pm 1.92	166.0 \pm 3.80 ***	123.0 \pm 2.55 ***	79.0 \pm 1.58 ***
4	90.0 \pm 2.55	91.0 \pm 2.24	159.2 \pm 4.44 ***	128.2 \pm 4.15 ***	93.8 \pm 2.59 **

Comparisons were made between: (*) Group 1 and group 2, 3, 4, ; The symbol represent statistical significance. *P < 0.05, **P< 0.01 and ***P<0.001. Four groups of glucose-induced rats were administered with 80 mg of plant extracts *A. secundiflora* (Group 2), *U. dioica* (Group 3), and *A. indica* (Group 4) and 0.9% NaCl as control (Group 1). Change in the blood glucose levels was studied at 0, 30, 60, 90 and 120 minutes.

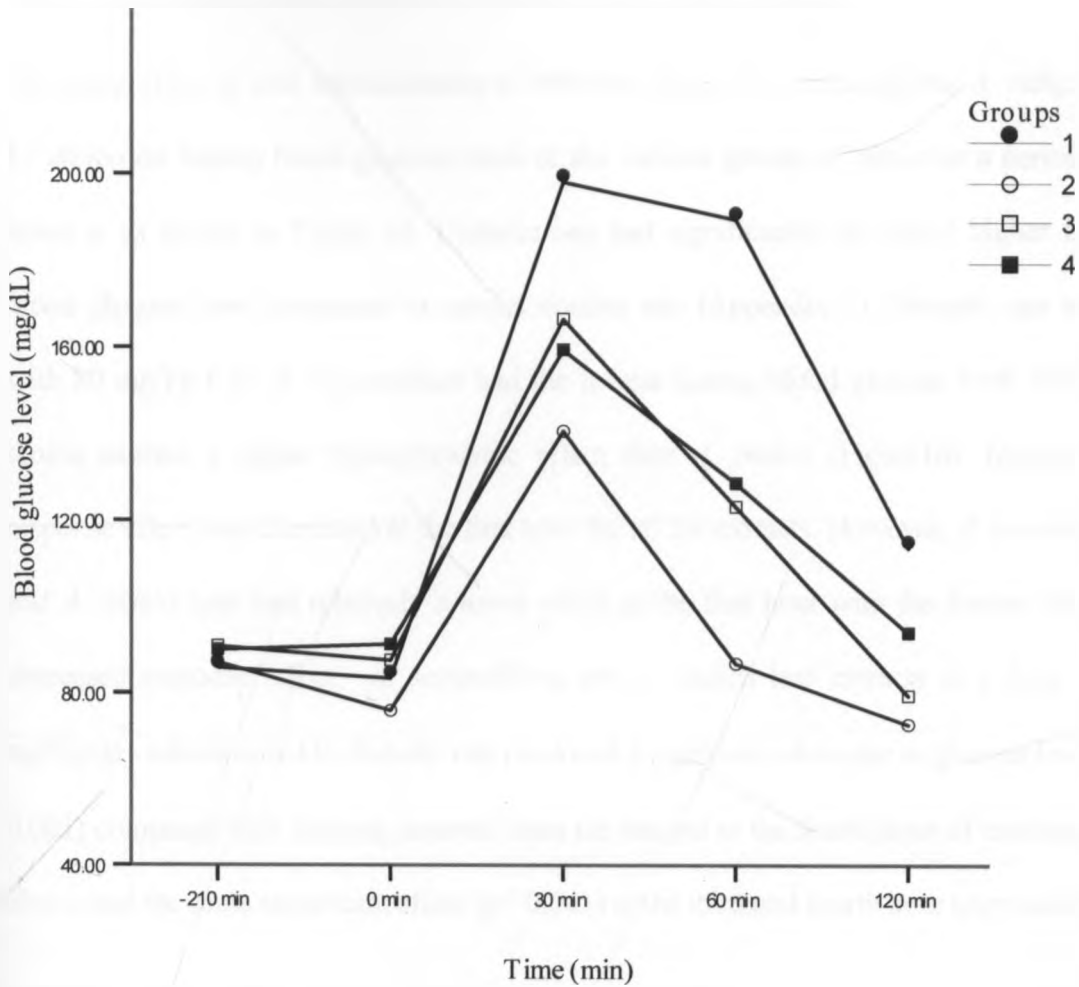


Figure 15: The hypoglycaemic effects of ethanol extracts of *A. secundiflora*, *A. indica* and *U. dioica* on glucose induced hyperglycaemia in normal rats. The rats were fasted overnight. 210 min before the oral administration of glucose (3 g/kg b.w.), four groups of rats were administered with 80 mg b.w. of plant extracts *A. secundiflora* (Group 2), *U. dioica* (Group 3), and *A. indica* (Group 4) and 0.9% NaCl as control (Group 1). Change in fasting blood glucose levels was studied at 0, 30, 60, 90 and 120 minutes. n=5 for each group.

4.5 Acute effect of the effective dose of leaf extracts of the *A. secundiflora*, *A. indica* and *U. dioica* plants on fasting blood glucose level in diabetic rats

The acute effect of oral administration of effective doses of *A. secundiflora*, *A. indica*, and *U. dioica* on fasting blood glucose level of the various groups of rats over a period of 4 hours is as shown in Figure 16. Diabetic rats had significantly ($p < 0.001$) higher fasting blood glucose level compared to normal control rats (Appendix 7). Diabetic rats treated with 80 mg/kg b.w. *A. secundiflora* had the lowest fasting blood glucose level whilst *U. dioica* exerted a higher hypoglycaemic effect than *A. indica* (Figure 16). Notably, the response effect was decreased at the first hour for all the extracts. However, *A. secundiflora* and *A. indica* both had relatively a lower effect at the first hour with the former showing decreased response effect. *A. secundiflora* and *A. indica* leaf extracts at a dose of 80 mg/kg/day administered to diabetic rats produced a significant decrease in glucose level ($p < 0.001$) compared with diabetic controls from the second to the fourth hour of treatment. *U. dioica* had the same significant effect ($p < 0.001$) at the third and fourth hour (Appendix 7).

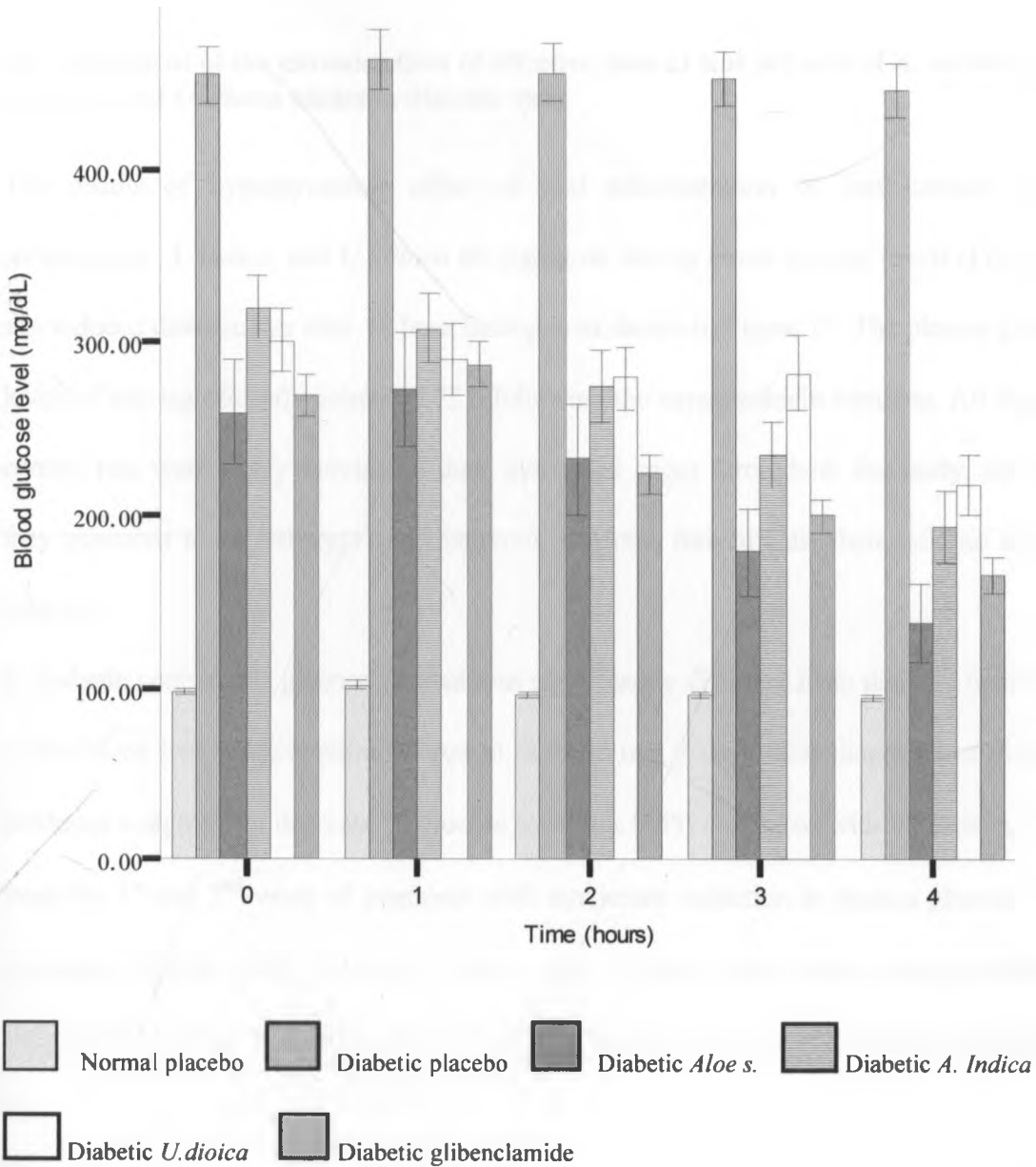


Figure 16: Acute effect of oral administration of effective doses of *A. secundiflora*, *A. indica*, and *U. dioica* on fasting blood glucose level in diabetic-induced rats. Three groups consisting of diabetic rats were administered with 80 mg/kg of plant extracts *A. secundiflora* (group 3), *A. indica* (group 4), *U. dioica* (group 5) and the fourth and the fifth groups consisting of diabetic rats were given placebo (as control, group 2), and glibenclamide 5 mg/kg (positive control, group 6). Group 1 consisted of non-diabetic normal rats. Change in the blood glucose levels were studied at 0, 1, 2, 3, 4 hours. Data are mean \pm SD. n=7 in all groups.

4.6 Assessment of the chronic effect of effective dose of leaf extracts of *A. secundiflora*, *A. indica* and *U. dioica* plants in diabetic rats

The results of hypoglycaemic effect of oral administration of leaf extracts of *A. secundiflora*, *A. indica*, and *U. dioica* 80 mg/kg on fasting blood glucose levels (FBGL) of stz- induced diabetic rats after 18 hour fasting is as shown in Figure 17. The plasma glucose levels of rats significantly increased 72 h following the streptozotocin injection. All diabetic control rats were freely moving in their individual cages throughout the study, although they appeared to be lethargic and displayed restricted movements, there was no sign of infection.

In diabetic control rats glucose content was significantly different from diabetic treated. *A. secundiflora* leaf extracts administered to diabetic rats 7 days after diagnosis of diabetes produced a significant decrease in glucose level ($p < 0.05$) compared with diabetic controls from the 1st and 2nd week of treatment with maximum reduction in plasma glucose level witnessed during week 3. Both *U. dioica* and *A. indica* leaf extracts also produced a significant decrease in glucose level ($p < 0.05$) compared with diabetic controls (Appendix 9).

This observation was prominent and the plasma glucose concentration appeared to be normalizing at 5 weeks treatment for *A. secundiflora* and these were stabilized downward throughout the treatment for all extracts (6weeks). The increased levels of blood glucose in treated diabetic treated rats were reverted back to near normal levels after 42 days treatment. Upon the end of treatment, there was no reversion back to diabetes for these rats. The blood glucose for *A. secundiflora* was similar to that of normal control (Figure 17). The extract treatment also resulted in a significant decrease in the amount of water taken (Figure 20). *A.*

indica and *A. secundiflora* extracts were observed to be more effective than glibenclamide in restoring the blood glucose values.

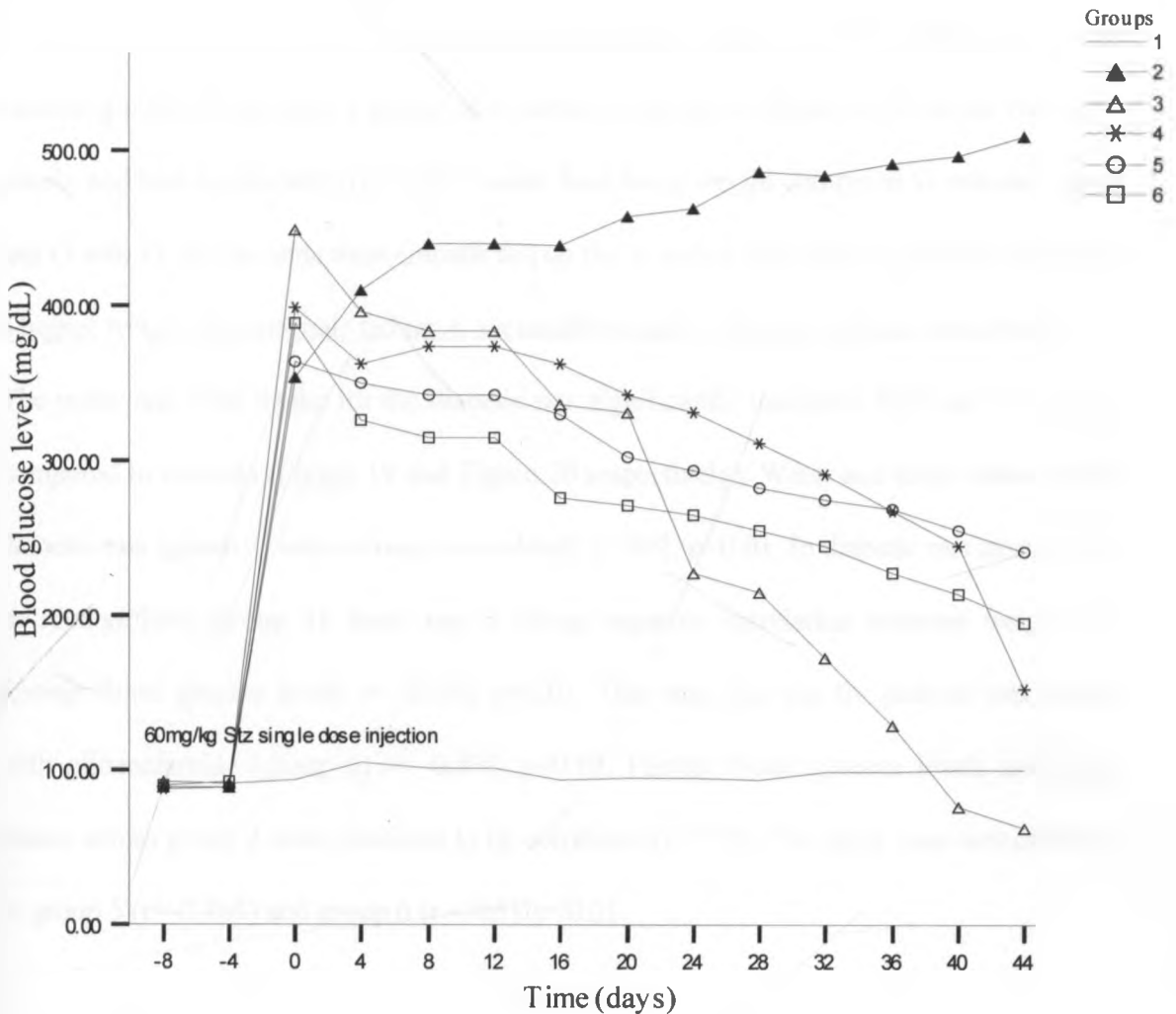


Figure 17: Chronic effect of oral administration of effective doses of *A. secundiflora*, *A. indica* and *U. dioica* on fasting blood glucose level in diabetic-induced rats. Four groups consisting of diabetic rats were administered with 80 mg/kg of plant extracts *A. secundiflora* (Group 3), *A. indica* (Group 4), *U. dioica* (Group 5) and placebo as control (Group 2). The fifth set consisting of diabetic rats was administered glibenclamide (positive control, Group 6). Group 1 consisted of non-diabetic normal rats fed on placebo. Change in the blood glucose levels was studied after every four days. n=7 in all groups.

4.7 Effects of *A. secundiflora*, *A. indica* and *U. dioica* leaf extracts on body weight of diabetic rats

Mean body weights of all animals at the start of the study are presented in Table 4. The initial body weights were similar in normal and diabetic groups. The % weight gain of the various groups of rats over a period of 6 weeks is shown in Figure 18. Diabetic rats grew poorly and had significantly ($p < 0.001$) lower final body weight compared to normal control rats (Table 4). At the same time animals fed on the *A. indica* leaf extract (group 4) exhibited a higher weight than animals fed on *A. secundiflora* and *U. dioica* extracts respectively.

The water and food intake for the diabetic rats significantly increased from day 1 to day 7 compared to controls (Figure 19 and Figure 20 respectively). Water and food intake for the diabetic rats (group 2) were strongly correlated, $r = .992$, $p < 0.01$. In diabetic rats treated with *A. secundiflora* (group 3), there was a strong negative correlation between weight and fasting blood glucose levels $r = -0.924$, $p < 0.01$. This was also true for diabetic rats treated with glibenclamide (group 6) $r = -0.869$, $p < 0.01$. Fasting blood glucose levels and water intake across group 2 were observed to be correlated ($r = .539$). The same case was observed in group 5 ($r = -0.463$) and group 6 ($r = .765$), $p < 0.01$.

Table 4: Effects of the ethanol extracts of *U. dioica*, *A. indica* and *A. secundiflora* on body weight in diabetic rats over a period of 6 weeks

Group treatment	BW (g)					
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
I Normal Control	183.14 ±9.04	212.94 ±9.03	244.70 ±18.71	277.29 ±8.16	294.71 ± 11.24	314.71 ±15.94
II Diabetic Control	181.93 ±9.30	162.36 ±9.38 ***	135.43 ±10.44 ***	106.98 ±11.83 ***	109.44 ± 6.39 ***	104.94 ± 6.72 ***
III <i>A. secundiflora</i>	185.71 ±5.59	198.86 ±6.41 **	218.86 ±9.03 **	233.86 ±20.22 ***	256.17 ± 10.83 ***	273.50 ±16.43 ***
IV <i>A. indica</i>	183.00 ±8.64	204.71 ±11.10	233.86 ±22.09	253.17 ±13.72 **	273.20 ± 15.96 *	289.20 ±15.74 *
V <i>U. dioica</i>	184.00 ±10.41	191.71 ±10.75 **	218.40 ±14.22 *	232.20 ±11.56 ***	247.40 ± 15.55 ***	268.60 ±14.15 ***
VI Glibenclamide mide	182.57 ±7.87	204.57 ±10.97	223.29 ±8.65 *	223.00 ±10.13 ***	227.29 ± 9.11 ***	242.71 ±12.18 ***

Comparisons were made between: (*) Group I and group II, III, IV, V; The symbol represent statistical significance. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$. Four groups consisting of STZ-induced diabetic rats were administered with 80 mg/kg b.w. of plant extracts and placebo as control (Group II). Group 1 consisted of non-diabetic normal rats. The sixth group of diabetic rats was administered glibenclamide (positive control). Values shown represent means ± S.D for at least 7 animals in each group.

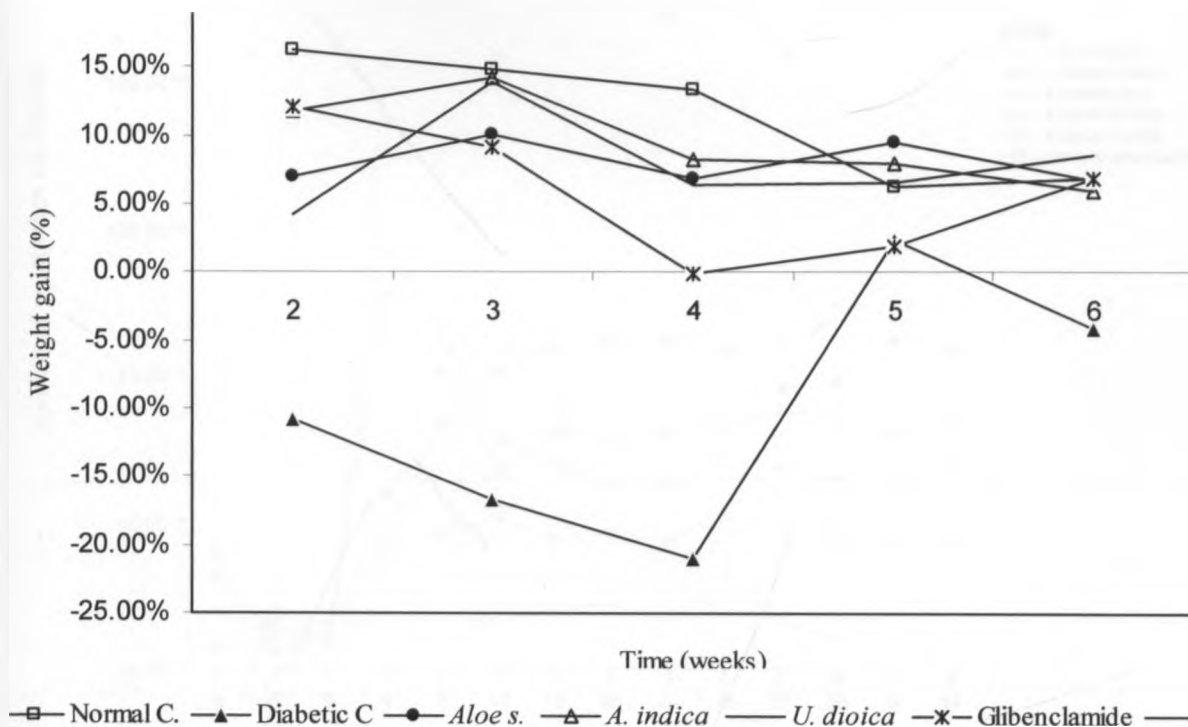


Figure 18: Effects of the ethanol leaf extracts of *U. dioica*, *A. indica* and dried leaf sap of *A. secundiflora* on % weight gain of rats over a period of 6 weeks. Normal C. (Non-diabetic control rats fed on placebo); Diabetic C. (Diabetic control rats fed on placebo); *Aloe s.* (Diabetic induced rats fed on *A. secundiflora*); *A. indica* (Diabetic induced rats fed on *A. indica*); *U. dioica* (Diabetic induced rats fed on *U. dioica*); Glibenclamide (Diabetic induced rats fed on glibenclamide). $n=7$ in all groups.

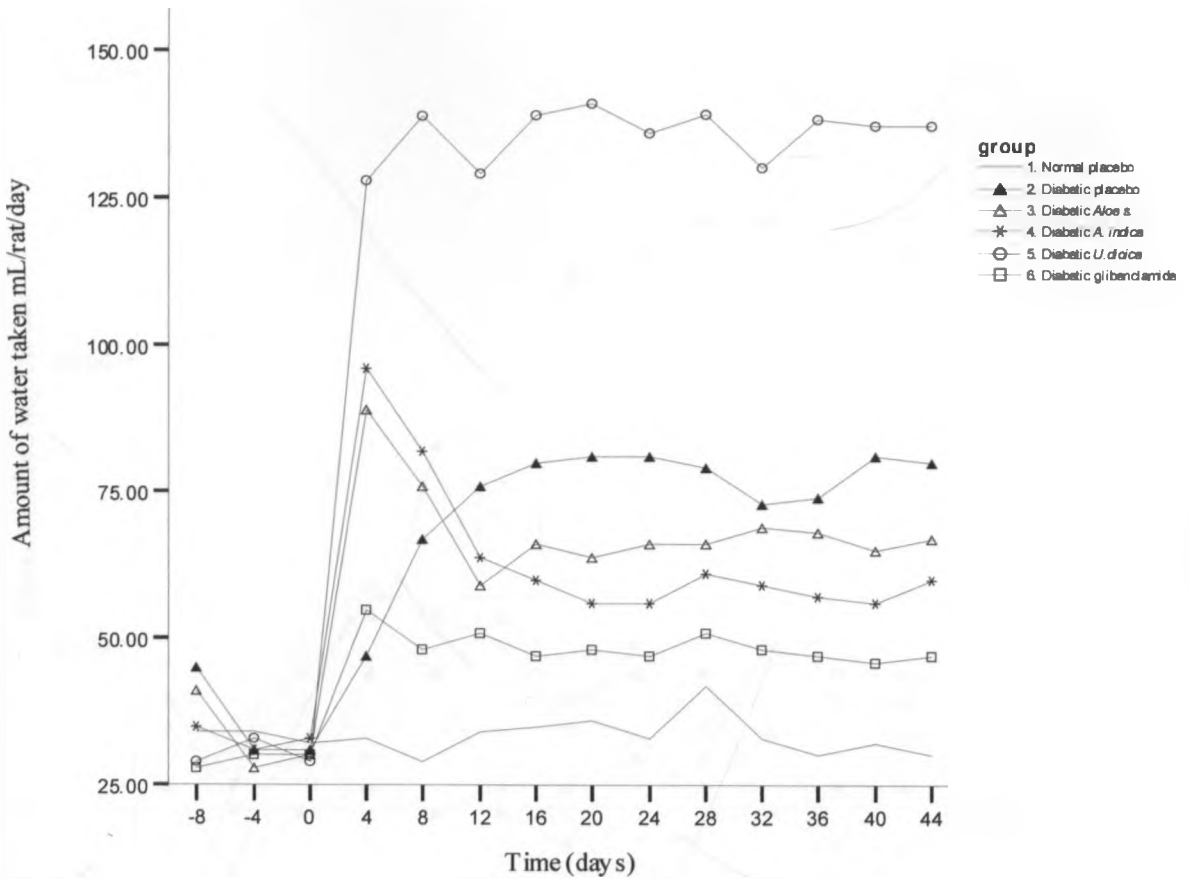


Figure 19: Amount of water taken by stz- induced diabetic rats orally fed on *U. dioica*, *A. indica* and *A. secundiflora* extracts over a period of 6 weeks. Normal C. (Non-diabetic control rats fed on placebo); Diabetic C. (Diabetic control rats fed on placebo); *Aloe s.* (Diabetic induced rats fed on 80 mg/kg b.w. *A. secundiflora*); *A. indica* (Diabetic induced rats fed on 80 mg/kg b.w. *A. indica*); *U. dioica* (Diabetic induced rats fed on 80 mg/kg b.w. *U. dioica*); Glibenclamide (Diabetic induced rats fed on 5 mg/kg glibenclamide). n= 7 in all groups.

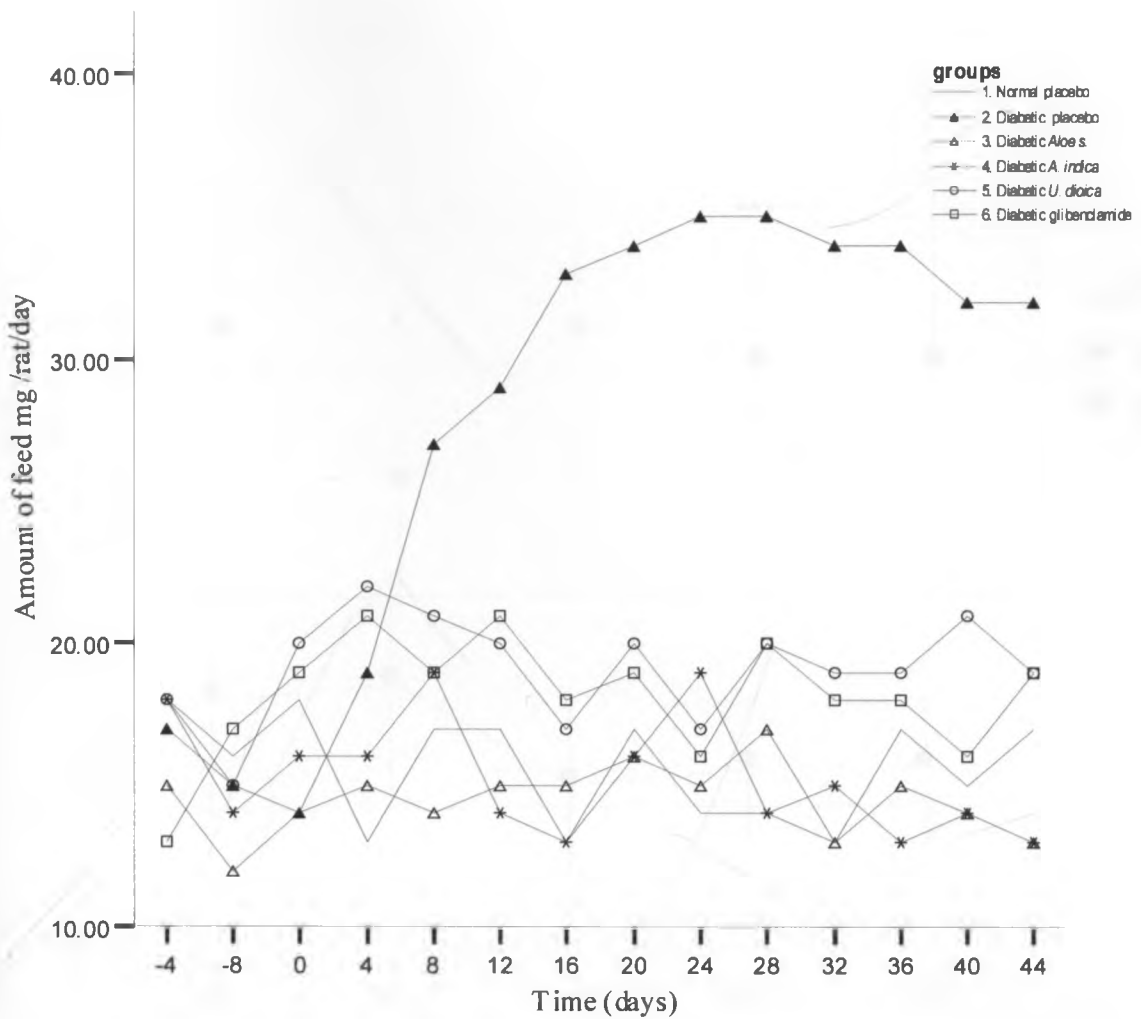


Figure 20: The effect of oral administration of *A. secundiflora*, *A. indica* and *U. dioica* on feed intake in stz- induced -diabetic rats. Normal C. (Non-diabetic control rats fed on placebo); Diabetic C. (Diabetic control rats fed on placebo); *Aloe s.* (Diabetic induced rats fed on 80 mg/kg b.w. *A. secundiflora*); *A. indica* (Diabetic induced rats fed on 80 mg/kg b.w. *A. indica*); *U. dioica* (Diabetic induced rats fed on 80 mg/kg b.w. *U. dioica*); Glibenclamide (Diabetic induced rats fed on 5 mg/kg glibenclamide). n= 7 in all groups.

4.8 Effect of *U. dioica* leaf extract on urine output in normal rats

As shown in Figure 21, significant differences were observed between urine volume levels of normal and control animals in the course of the experiment. *U. dioica* significantly increased the urine volume in normal rats in comparison with the control group fed on placebo, which had no effect on the urine volume levels.

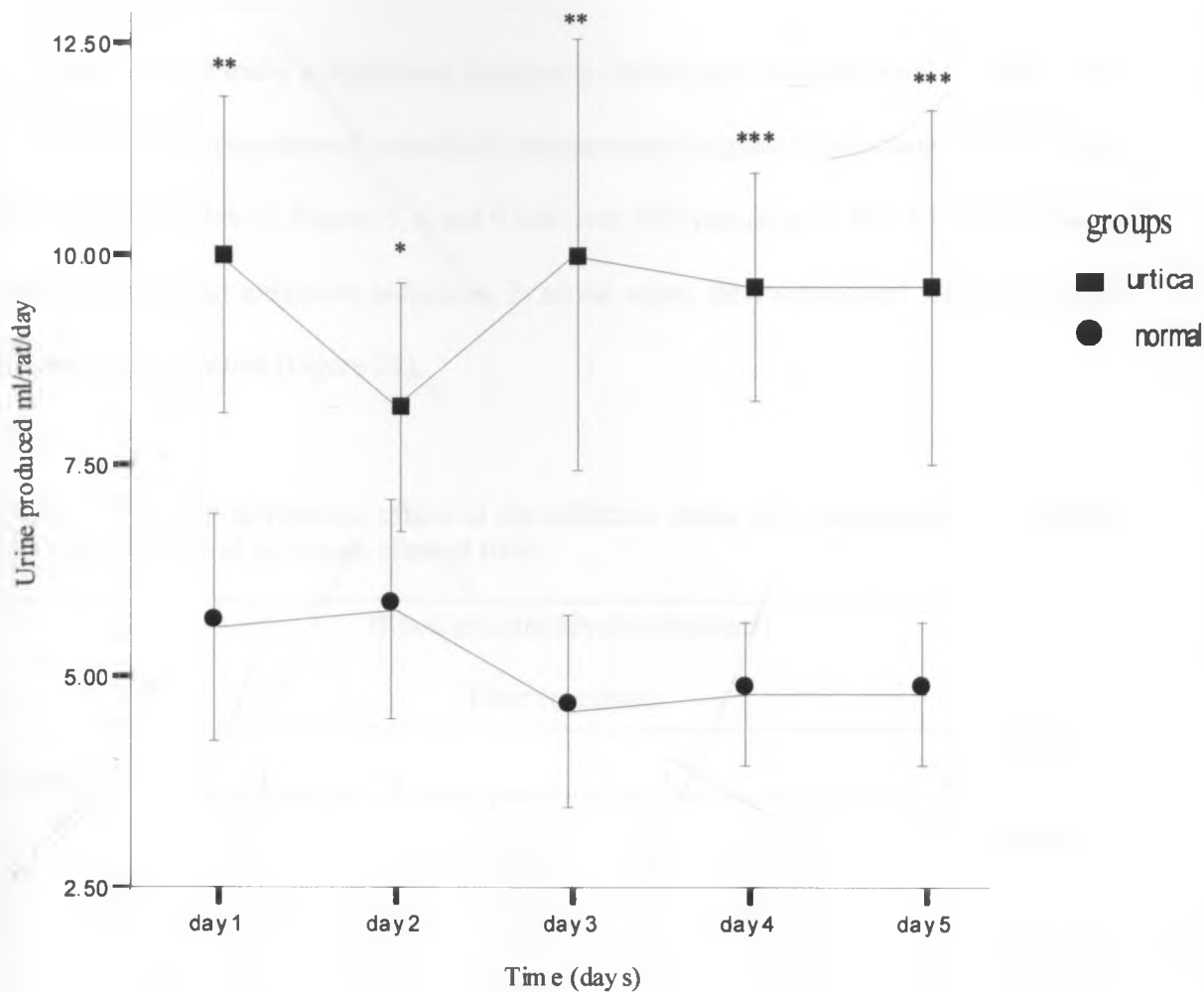


Figure 21: The effect of ethanol leaf extract of *U. dioica* leaves on urine output in normal rats. Comparisons were made between: (*) Group 1 and group 2 ; The symbol represents statistical significance. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$. One group of normal rats was administered with 80 mg/kg b.w. of *U. dioica* plant extracts and another with placebo as control. Change in the urine volume levels were studied for 5 days.

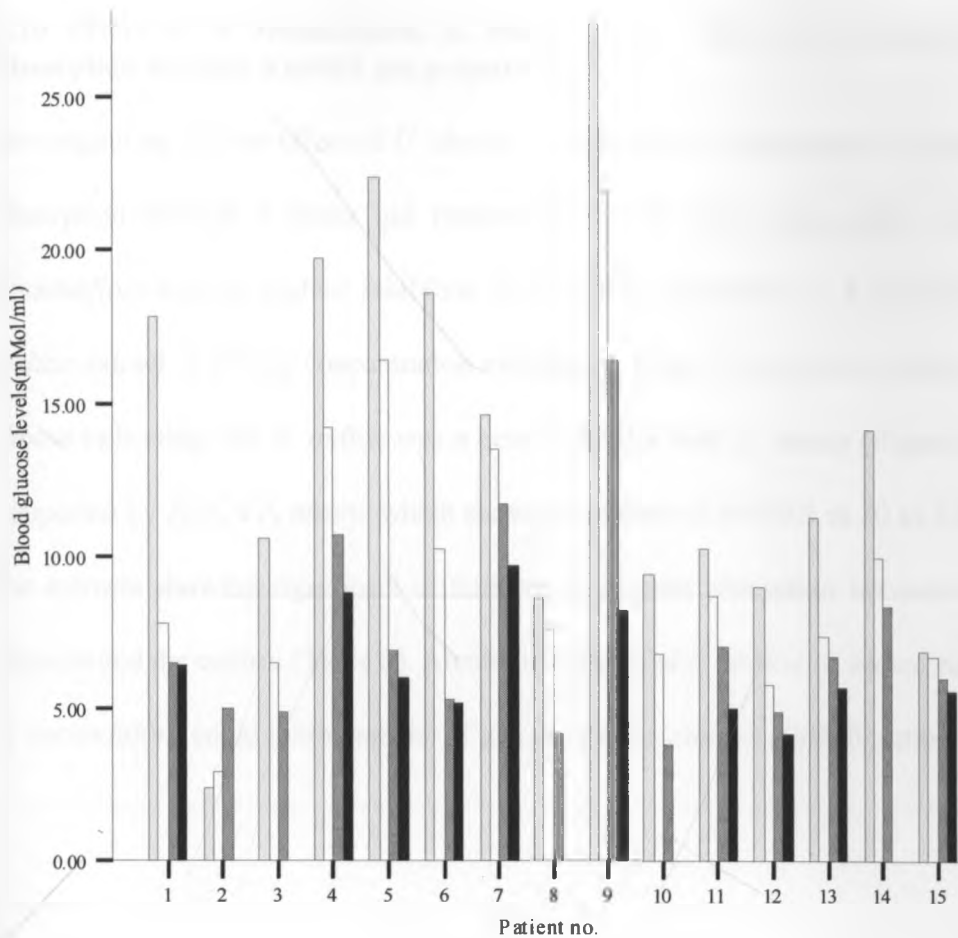
4.9 Assessment of the effective dose of *A. secundiflora* extract on blood glucose levels in human clinical trials

In human clinical trials, a significant decrease in blood glucose is observed throughout the study. Six (6) patients showed remarkable improvement in glucose reduction within the first three months (Table 5). Patient 5, 6 and 9 had over 70% reduction in blood sugar. Although Patients 10-15 had minimum reductions in blood sugar, their subsequent levels were kept regulated at a constant (Figure 22).

Table 5: The hypoglycaemic effect of the effective doses of *A. secundiflora* on fasting blood glucose level in human clinical trials

Patient	Blood glucose level (mMol/ml)					
	Time (months)					
	1	2		3		4
1	17.8	7.8	(56.1%)*	6.5	(63.5%)*	6.4 (64.04%)*
2	2.4	2.9	(-20.8%)*	5.0	(-108.3%)*	
3	10.6	6.5	(38.7%)*	4.9	(53.8%)*	
4	19.7	14.2	(27.9%)*	10.7	(45.7%)*	8.8 (55.3%)*
5	22.4	16.4	(26.8%)*	6.9	(69.2%)*	6.0 (73.2%)*
6	18.6	10.2	(45.2%)*	5.3	(71.5%)*	5.2 (72.0%)*
7	14.6	13.5	(7.5%)*	11.7	(19.9%)*	9.7 (33.6%)*
8	8.6	7.6	(11.6%)*	4.2	(51.2%)*	
9	27.4	21.9	(20.1%)*	16.4	(40.2%)*	8.2 (70.1%)*
10	9.4	6.8	(27.7%)*	3.8	(59.6%)*	
11	10.2	8.7	(14.7%)*	7.0	(31.4%)*	5.0 (51.0%)*
12	9.0	5.8	(35.6%)*	4.9	(45.6%)*	4.2 (53.3%)*
13	11.3	7.4	(34.5%)*	6.7	(40.7%)*	5.7 (49.6%)*
14	14.2	10.0	(29.6%)*	8.4	(40.9%)*	
15	6.9	6.7	(2.9%)*	6.0	(13.0%)*	5.6 (18.8%)*

*Percentage fall in blood glucose concentration compared with initial values. Blank cells means patients did not turn up.



[] 1st month [] 2nd month [] 3rd month [] 4th month

Figure 22: The hypoglycaemic effect of *A. secundiflora* 80 mg/kg b.w. on fasting blood glucose level in human clinical trials. Fifteen patients, aged between 27-82 years were enrolled for the study. They were administered formulated Aloe extracts 80 mg/Kg. Their blood glucose level was monitored until it stabilized.

4.10 Effect of *A. secundiflora*, *A. indica* and *U. dioica* leaf extracts on glucose absorption through a rabbit gut preparation

Investigations into the effect of *U. dioica*, *A. indica* and *A. secundiflora* extracts on glucose absorption through a rabbit gut preparation carried out in this study showed that *A. secundiflora* had the highest inhibition % on glucose absorption in a rabbit preparation. *A. indica* extract at 40 mg concentration exhibited a much lower glucose absorption than *U. dioica* indicating that *A. indica* was a better inhibitor than *U. dioica* (Figure 23). This was supported by ANOVA results which showed p-values of $p=0.001$ at 30 to 120 mins for all the extracts showing significant differences in glucose absorption between the means of extracts and the control (Table 6). Alcoholic extracts of *U. dioica*, *A. indica* and dried sap of *A. secundiflora* could inhibit uptake of glucose by the isolated rabbit intestine.

Table 6: Effects of ethanol extracts of *A. indica*, *U. dioica*, *A. secundiflora* on glucose uptake on intestinal glucose absorption in isolated intestinal segments of rabbit (decrease in the glucose concentration of medium indicate the absorption of glucose through intestine)

Time(min)	Glucose conc. (mg/dL) in the medium														
	Group 1			Group 2			Group 3			Group 4			Group 5		
0	194.48	±0.4		196.60	±0.3		195.44	±0.3		195.84	±0.3		193.68	±0.4	
15	184.80	±1.0	(5.0%)*	189.76	±0.3	(3.5%)*	188.16	±0.5	(3.7%)*	190.36	±0.6	(2.8%)*	185.08	±0.6	(4.4%)*
30	155.84	±1.5	(19.9%)*	169.96	±0.7	(13.6%)*	166.56	±0.8	(14.8%)*	162.48	±0.8	(17.0%)*	173.96	±1.2	(10.2%)*
60	140.60	±1.4	(27.7%)*	159.96	±1.0	(20.2%)*	156.28	±0.7	(20.0%)*	146.48	±0.5	(25.2%)*	165.60	±1.2	(14.5%)*
90	124.08	±1.3	(36.2%)*	151.12	±0.9	(23.1%)*	143.24	±0.6	(26.7%)*	135.56	±0.7	(30.8%)*	158.20	±1.2	(18.3%)*
120	109.40	±1.3	(43.8%)*	142.24	±1.2	(27.7%)*	133.76	±1.0	(31.6%)*	125.40	±0.9	(36.0%)*	152.20	±1.2	(21.4%)*

* Percentage fall in glucose concentration of the medium with initial values.

Five sets of rabbit jejunum segments were incubated with 40 mg of plant extracts, *A. secundiflora* (Group 2) *A. indica* (Group 3) *U. dioica* (Group 4) and buffer as control (Group 1). The fifth set was placed in 100µg of acarbose (positive control, Group 5). Change in the glucose concentration in the medium was studied at 0, 30, 60, 90 and 120 minutes. Data are in mean ± SD.

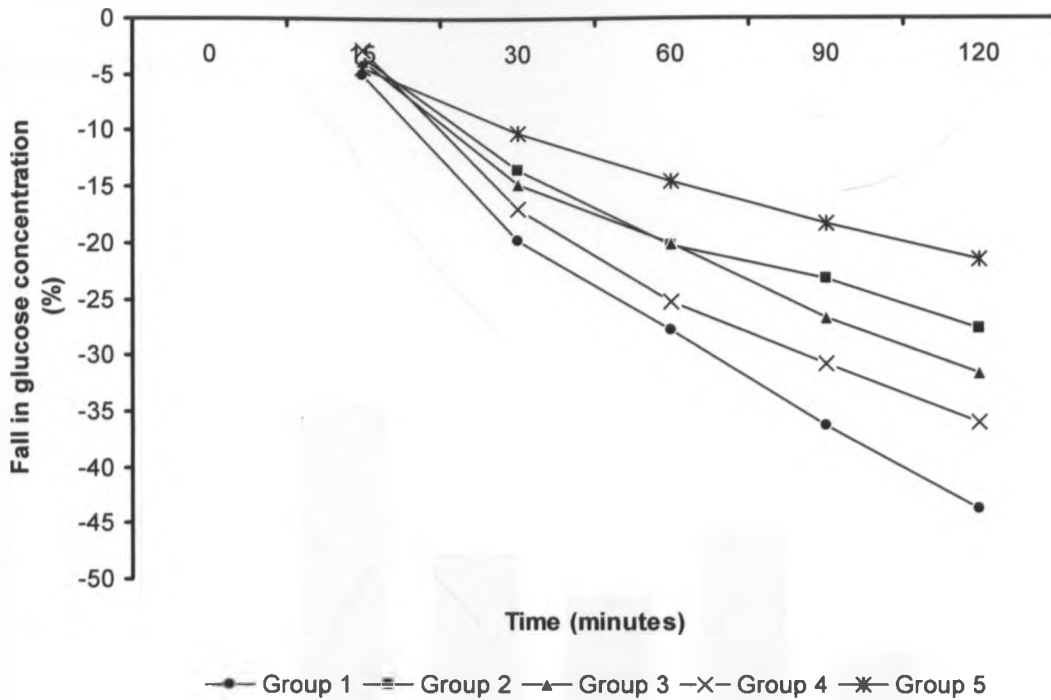


Figure 23: Effects of leaf extract of *A. secundiflora*, *A. indica*, and *U. dioica* on glucose uptake on intestinal glucose absorption in isolated intestinal segments of rabbit (decrease in the glucose concentration of medium indicate the absorption of glucose through intestine). Group 1 consisted of buffer as control, Group 2 *A. secundiflora*, Group 3 *A. indica*, Group 4 *U. dioica* and Group 5 of acarbose, a positive control. Data shows percentage fall in glucose concentration of the medium with initial values.

4.11 Histopathologic examinations

An elevated kidney weight was observed in diabetic rats compared to controls (Figure 24) suggesting that STZ-induced diabetes produced renal hypertrophy. Histologically, the kidneys section of STZ-diabetic control rats showed marked multifocal clarifications, vacuolations and abundance of mucopolysaccharide in diabetic rats' kidneys. Although, in diabetic groups (group 2, 3, 4, 5 and 6) the numbers of islets were observed to be less than the normal (group 1), the diabetic control (group 2) was observed to have the lowest islet number. In the diabetic glibenclamide treated group

(6), the size of the islets was smaller than the normal control and the extract diabetic treated groups.

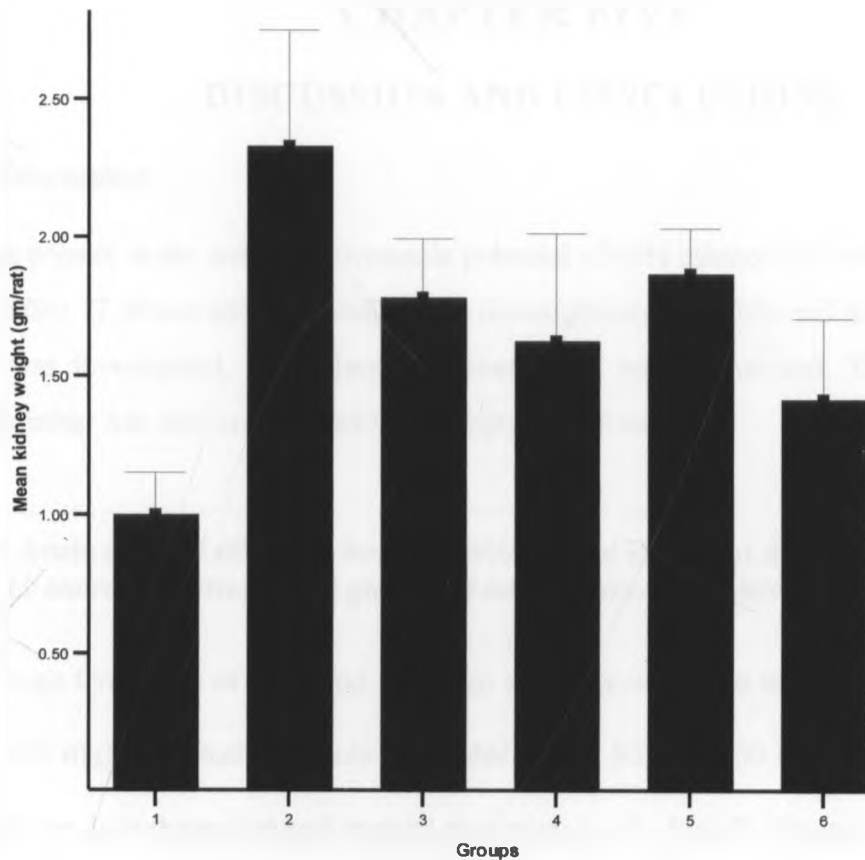


Figure 24: The effect of *A. secundiflora*, leaf ethanol extracts of *A. indica* and *U. dioica* on kidney weights of STZ- induced diabetic rats after six weeks of treatment. 1- Non-diabetic control rats fed on placebo; 2- Diabetic control rats fed on placebo; 3-Diabetic induced rats fed on 80 mg/kg b.w. *A. secundiflora*; 4-Diabetic induced rats fed on 80 mg/kg b.w. *A. indica*; 5-Diabetic induced rats fed on 80 mg/kg b.w. *U. dioica* and 6 consisted of Diabetic induced rats fed on 5 mg/kg glibenclamide. n= 7 in all groups.

CHAPTER FIVE

DISCUSSION AND CONCLUSIONS

5.1 Discussion

In the present study, antihyperglycaemic potential of 60% ethanol (1:3 w/v) leaf extracts of *A. indica*, *U. dioica* and *A secundiflora* in normoglycaemic rabbits and stz-induced diabetic rats was investigated. Oral glucose tolerance test was determined. The dose-response relationship was also investigated in normoglycaemic rabbits.

5.1.1 Acute effect of different doses of 60% ethanol extract of *A secundiflora*, *A. indica* and *U. dioica* on fasting blood glucose of normoglycaemic rabbits after 18 hour fasting

Although five levels of *U. dioica*, *A. indica* and *A. secundiflora* were tried (10, 20, 40, 80, and 100 mg) the effect was only detectable in 40, 80, and 100 mg. The hypoglycaemic effect was dose-dependent and reached maximum level within 3–4 hours. At 10 and 20 mg level, there was no significant alteration. This may be as a result of the fact that some of the hypoglycaemic components in the ethanol extract at lower doses did not show significant effect. Administration of 40 mg extract of *A. secundiflora*, *A. indica* and *U. dioica* produced a hypoglycaemic effect within the first, second and third hour respectively in the normoglycaemic rabbits. Notably, there was a marked plasma glucose increase in the first 30 minutes after ingestion of *A. secundiflora*. *Aloe sp* contains monosaccharides and polysaccharides (Solov'era, 1958). The marked plasma glucose increase may have been brought about by the digestion and absorption of these carbohydrates, causing an increase in the workload of β -cells of pancreatic islets.

Three hours after extract administration, animals fed on 100 mg/kg of *U. dioica* extracts were observed to be weak, dizzy and hungry. These animals also experienced muscle twitching movements accompanied by the rabbit's fur increasingly standing on end. The hypoglycaemic effect of *U. dioica* extract may have caused the blood sugar levels to drop below normal, triggering the feedback system governing blood sugar in the rabbit. A combination of limited sugar in the cells and energy deficiency in the muscles could have signalled the body's effector organs to produce heat therein raising the body temperature.

Similar studies showed that aqueous extract of *U. dioica*, aqueous leaf extracts of *A. indica* and various aloe constituents have anti-hyperglycaemic activity (Bnouham *et. al.*, 2003; Blitz *et. al.*, 1963; Ghannam *et. al.*, 1986) respectively.

5.1.2 Determination of chronic effect of graded doses of different ethanol extracts of *A. secundiflora*, *A. indica* and *U. dioica* in normoglycaemic rabbits.

In this study the duration taken for hypoglycaemic activity to be manifested differed with the various extracts and concentrations. The highest activity was shown by *A. indica* extract at 100 mg/kg treatment showing significant reduction from a mean of 102 mg/dl blood glucose level on day 0 to 79 mg/dl on the 30th day. The reduction was much higher as compared to that of the standard, glibenclamide. *U. dioica* had 85 mg/dl on the 30th day, reducing from a mean of 96 mg/dl on day 0, while *A. secundiflora* extract showed no change in fasting blood glucose level. In this study *A. secundiflora* showed acute and no chronic hypoglycaemic effect in normal rabbits, this could be an indication that the hypoglycaemic effect may be reversible.

The observed higher frequency and loosening of stool in animals fed on 100 mg/kg b.w. of *A. secundiflora* could be an indication that the dose was high. Excessive continuous consumption of higher doses of *A. secundiflora* may have stimulated colonic motility, augmented propulsion and accelerated colonic transit. This may have led to a reduction in

fluid absorption from the faecal mass. Studies show that *Aloe* leaf sap contains anthraquinone glycosides that are potent stimulant laxatives (Yongchaiyudha *et. al.*, 1996) which may have effected the laxative action observed in these animals. Other studies show that overuse of aloe, along with cardiac glycoside drugs, might potentiate diuretic-induced potassium loss and produce loss of fluid and electrolytes (De Smet, 2002). An indication as to why animals fed on 100 mg/kg b.w. *A. secundiflora* extract were observed to have higher water intake. The correct individual dose was settled at 80 mg/kg b.w. as this was the smallest amount required to produce a soft-formed stool and yet still, exerting hypoglycaemic effect. This dosage was therefore considered effective for all the extracts.

This study shows a substantial chronic hypoglycaemic activity of ethanol leaf extracts of *A. indica* and *U. dioica* after 30 days of treatment of normoglycaemic rabbits with the plant extracts. On the other hand, *A. secundiflora* leaf extract was ineffective in lowering the blood sugar of normoglycaemic rabbits, contrary to *A. indica*, *U. dioica* and glibenclamide supposing that their mechanisms of action are different.

5.1.3 The hypoglycaemic effect of *A. secundiflora*, *A. indica*, and *U. dioica* on glucose induced hyperglycaemia in normal rats

This investigation showed a high oral glucose tolerance potential of *A. secundiflora* extract. It also suggests that *A. indica* leaf extract may exert its effect differently or its mode of action could be prolonged. This was manifested by its moderate activity that was different from that of *A. secundiflora* and *U. dioica*. *Aloe vera* polysachharides are thought to release insulin from insulin stored granules by involving the closure of ATP-gated potassium channels and activation of voltage-gated calcium channels (Mukherjee *et. al.*, 2006). This may increase the serum insulin level, reduce blood glucose level thereby improving tolerance of glucose. Arborans A and B derived from *Aloe* polysaccharide is a

hypoglycaemic agent, Hikino *et. al.*, (1986) and may be responsible for improving oral glucose tolerance. Urticin a hypoglycaemic component in nettle (Oliver and Zahland, 1979) may have a similar mode of action. Urticin lowers the blood sugar concentration in hyperglycaemic rabbits. This might be one mechanism in which nettle extract regulates glucose homeostasis in glucose-loaded rats. On the other hand mechanism of action of *A. indica* involves blocking the reduction in the peripheral utilization of glucose and glycogenolysis (Chattopadhyay, 1996). Chattopadhyay (1999) also found hydroalcoholic leaf extracts of *A. indica* to improve oral glucose tolerance and lower blood sugar levels in a dose-dependant manner in normal rats. This study suggests that *A. secundiflora*, *A. indica* and *U. dioica* (80 mg/kg) given orally inhibited the increase in blood glucose of normal rats differently.

5.1.4 Acute effect of the effective dose of leaf extracts of the *A. secundiflora*, *A. indica* and *U. dioica* plants on fasting blood glucose level in diabetic rats

In the treated diabetic-induced rats highest drop in fasting blood glucose levels was shown by *A. secundiflora* followed by *A. indica* during the first and second hour. The hypoglycaemic component in the above extracts might act faster than *U. dioica* in this diabetic model. Notably, the blood glucose levels of stz induced-diabetic control rats fed on placebo maintained high fasting blood glucose levels throughout the four hour period. In this study *A. secundiflora* leaf extracts showed acute hypoglycaemic activity on stz - induced diabetic rats, the effectiveness being enhanced in comparison with glibenclamide. This study indicates that *A. secundiflora* leaves could be useful in the treatment of non-insulin dependent diabetes mellitus.

5.1.5 Assessment of the chronic effect of effective dose of *A. secundiflora*, *A. indica* and *U. dioica* leaf extracts in diabetic rats.

There was significant improvement in the fasting blood glucose levels of treated diabetic rats when compared with untreated diabetic rats. The extracts appeared to be more effective than glibenclamide in controlling the fall in fasting blood glucose levels. This was supported by ANOVA results which showed p-values of $p=0.001$ for all the three extracts indicating significant differences in means of fasting blood glucose levels (Appendix 9).

The extracts notably stopped progress in hyperglycaemia development which was not realized in the diabetic control hence rising to the highest peak at day 30 of 508 mg/dL. The improvement of hyperglycaemic state associated with stz-induced diabetes and *A. secundiflora* treatment in the present investigation could be attributed to the plant's antidiabetic action. Extracts of medicinal plants either modulate glycolysis, Krebs cycle, gluconeogenesis, HMP shunt pathway, glycogen synthesis and their degradation, cholesterol synthesis, metabolism and absorption of carbohydrates, and or synthesis and release of insulin. (Arulrayan *et. al.*, 2007). *A. secundiflora* may bring about its hypoglycaemic action by maintaining the glucose homeostasis through control of the carbohydrate metabolizing enzymes (Rajasekaran *et. al.*, 2004), or by increasing the pancreatic secretion of insulin from the β -cells of islets of Langerhans and potentiating its action (Mukherjee *et. al.*, 2006) through glucose metabolism enhancement (Boudreau and Beland, 2006). This is primarily the same mechanism by which glibenclamide acts as an oral antidiabetic agent.

This findings showed that *U. dioica* controls the increase in the concentration of glucose by either increasing insulin content of blood sera as nettle extract may mimic insulin actions (Czech and Lynn, 1973), or the plant may also inhibit the activity of α -glucosidase, one of the key enzymes of carbohydrate digestion. Quercetin, kaempferol, and glucoquinone present in *U. dioica* may act to inhibit the activity of α -glucosidases (Onal *et. al.*, 2005).

Inhibition of α -glucosidase may act as an effective treatment of *Diabetes mellitus*. These may indicate mechanisms in which nettle extract regulates the glucose homeostasis in diabetic rats. On the other hand, while *U.dioica* may be fast acting, *A.indica* seems to reduce blood sugar levels slowly over a prolonged period of time. This may be partly because of its mode of action. *A. indica* may increase peripheral glucose utilization by blocking the action of epinephrine on glucose metabolism, (Chattopadhyay, 1996). Hypoglycaemic activities of *A. indica* have been attributed to Nimbidin, a constituent of neem plant which exerts significant effect on blood glucose levels. Chattopadhyay (1999), in related studies also revealed hydroalcoholic leaf extracts of *A. indica* lowered blood sugar levels in streptozotocin induced diabetic model rats. Glibenclamide and aloes also exert the same effect in streptozotocin – induced mice (Rajasekaran *et al*, 2004).

In conclusion, investigations into the treatment of diabetic rats with 60% ethanol extract of *A. secundiflora*, *A.indica* and *U. dioica* carried out in this study showed that chronic administration (80 mg/kg b.w. once daily for 6 weeks) of *A. indica*, *U. dioica* and *A. secundiflora* leaf sap powder showed significant hypoglycaemic effect in stz-induced diabetic rats. Thus, this study confirms the ethnopharmacological use of *A. secundiflora*, *U. dioica* and *A. indica* in managing the hyperglycaemic condition found in diabetes.

5.1.6 Effects of *A. secundiflora*, *A. indica* and *U. dioica* leaf extracts on body weight of diabetic rats

The live body weight of the streptozotocin diabetic rats reduced over time. There was a marked increase in food and water intake for the diabetic rats from day 1 to day 7. Similarly, increased urination was also observed. The later may have been the body attempts to clear the sugar build- up in the blood resulting from insufficient insulin in the body. Loss of glucose by kidneys through urine caused a large amount of water to be lost,

causing dehydration, unrelenting thirst and too much water taking (polydipsia). The general feeling of great hunger for the animals (Arky, 1979) may indicate that the large amount of glucose in the blood may not have been available for the appetite regulating cells at the feeding and satiety centres of the hypothalamus, leading to excessive eating. In turn this results to stimulation of protein breakdown to provide amino acids for gluconeogenesis resulting in muscle wasting and weight loss. The above results are in agreement with those of other workers. Bar-On *et. al.*, (1976) reported that a single intravenous injection of STZ (50 mg/kg) could cause decrease in body weight. In a similar study, Kavalali *et. al.*, (2003) reported that food and water intake amount was higher in diabetic groups than in the control group.

Administration of *A. secundiflora*, *A. indica* and *U. dioica* increases the body weight in streptozotocin diabetic rats. The ability of these extracts to protect body weight loss seems to be as a result of their ability to reduce hyperglycaemia thereby stabilizing the blood sugar and thus eliminating the symptoms of high blood sugar. Antidiabetic activity of *A. secundiflora*, *A. indica* and *U. dioica* plant extracts may in the long run, relieve diabetic symptoms and prevent its complication.

5.1.7 Effects of *U. dioica* leaf extract on urine output in normal rats

Administration of nettle 60% ethanol extract at 80 mg/kg b.w. via a gastric tube to rats increased urine output. This may be attributed to the presence of high mineral content in nettle. This is supported by the fact that there was increased water intake in the animals fed on extracts. This work is in agreement with early studies which showed that flavonoids and high potassium content may contribute to the herb's diuretic action, (Bradley, 1992). The diuretic effect of nettle in animals is accompanied by increased excretion of chlorides and urea. Tita *et al.* (1993) found that intraperitoneal administration of 500 mg/kg in rats

significantly increased both urinary excretion and K^+ concentration in the urine. Na^+ excretion was unaffected. Low nettle ethanol extract (1 g/kg b.w.) showed no significant diuretic effect in rats. Extractant solvent used, mode of administration and vehicle used, may affect the diuretic response of animals to nettle. This data however, indicates that further studies are necessary to clarify nettle's diuretic activities. The results of this investigation demonstrate a diuretic action of 80 mg/kg b.w. *U. dioica* that may indicate an effect on the animal's renal functioning.

5.1.8 Assessment of the effective dose of *A. secundiflora* leaf extract on blood glucose levels in human clinical trials

In the present study it was noticed that *A. secundiflora* leaf extract had a role in controlling the blood sugar level in diabetic patients. Except for two patients who complained of abdominal spasms and pain after the first single dose and were turned away, no side effect was noticed in the entire study. Patients were followed for a period of four months. All the patients turned up for regular follow up for the first three consecutive months. Five patients did not turn up for the fourth month. Possible explanation could be that the patients may have seen no reason to do so after their blood sugar stabilized to almost normal. The diabetics had been put on diet control after the 1st month. However, in one case of hyperglycaemia, hypoglycaemia developed after treatment with *A. secundiflora* (patient 12, Table 5) pointing to a need for constant blood glucose monitor for diabetics using the extract. Many secondary plant metabolites have been reported to possess antioxidant activity. The exact mechanism of *Aloe* spp is not known but its leaf extract's antioxidant property (Rajasekaran *et. al.*, 1995) may modulate oxidative stress in the diabetes patients contributing to their overall well-being. Oxidative stress results from an imbalance between radical –generating and radical scavenging systems.

Besides these results, in one case of hypoglycaemia (patient 2, Table 5) in which *A. secundiflora* extract was administered the blood glucose levels were observed to improve. This could be an exception to the rule or a case of variation in the patient that may be outside the known population or still the plant's mode of action could be varied. Aloes need be administered with caution as they can cause hyperglycaemia. These results are in agreement with those of other workers, (Ghannam *et. al.*, 1986; Okyar *et. al.*, 2001). This data indicates that 80 mg/b.w. of *Aloe secundiflora* extract when given to the diabetes patients shows substantial improvement (above 18.84% decrease) in their clinical profile.

5.1.9 Effect of *A. secundiflora*, *A. indica* and *U. dioica* extracts on glucose absorption through a rabbit gut preparation

The extract inhibits the glucose absorption through the intestine. This is indicated by the reduced glucose absorption from the medium. This is also true in the case of standard drug acarbose compared with the control. Nettle extracts could inhibit α -Glucosidase, a membrane-bound enzyme located at the epithelium of the small intestine, which catalyzes the cleavage of glucose from disaccharides and oligosaccharides. This action causes reduced intestinal glucose absorption via the brush border cell of the intestine. This may indicate one mechanism in which nettle extract regulates the glucose homeostasis in glucose-loaded rats. Suggesting that *U. dioica* in this case does not have a direct effect on insulin secretion or its sensitivity, but on the absorption of carbohydrates in the small intestine. Bnouham *et. al.*, (2003) has shown that nettle significantly inhibits glucose absorption in small intestines in rats under anesthesia.

Other studies indicate that alkaloids show a hypoglycaemic activity by inhibiting glucose transport through the intestinal epithelium (Pan *et. al.*, 2003). *A.indica* also contains β – Sitosterol, a steroid that may exhibit potent hypoglycaemic activity as saponins are known to possess potent hypoglycaemic activity (Rao and Gurfinkel, 2000). It could also be

speculated that the observed effect of *U. dioica* and *A. indica* leaves on glucose absorption might also be related to the presence of alkaloids and saponins respectively.

5.1.10 Histopathologic examinations

There was significant improvement in the nephrons of kidneys of treated STZ-induced diabetic rats when compared with untreated diabetic rats. The ethanol extracts were more effective than glibenclamide in controlling the recovery of renal morphology. The improvement of renal morphology associated with STZ-induced diabetes and *A. secundiflora* treatment in the present investigation could be attributed to its antidiabetic action resulting in alleviation of hyperglycaemia. Hyperglycaemia may cause damage to eyes, kidneys, nerves, heart and blood vessels. Administration of *A. indica* to stz- induced diabetic rats decreased the degree of tissue damage in these animals confirming the function of the extract on the protection of vital tissues including the pancreas, thereby reducing the causation of diabetes in rats.

These findings indicate that *U. dioica* also prevents the cellular damage of pancreas, as compared to glibenclamide. Stz has been found to induce free radical generation and cause pancreatic tissue injury (Schein and Loftus, 1968). Anti-oxidant and free radical scavenging properties of *U. dioica* leaves have been established by several studies (kanter *et. al.*, 2000). All structural changes in kidneys resulting from STZ administration in rats are due to altered metabolism in diabetes as streptozotocin does not possess any significant nephrotoxic potential (Floretto *et. al.*, 1998). This confirms the use of *A. secundiflora*, *A. indica* and *U. dioica* in improving the renal structural changes found in diabetes as was evident from the morphology of the kidney.

5.2 Conclusions

1. Ethanol extracts of *Azadirachta indica*, *Urtica dioica* and dried sap of *Aloe secundiflora* leaves (10, 20, 40, 80, and 100 mg/kg b.w.) have acute hypoglycaemic effects in normoglycaemic fasted rabbits. These extracts produced a profound and dose dependent hypoglycaemia. However the extracts have little or no significant effect on prolonged fasting blood glucose levels of normal rabbits.
2. Ethanol extracts of *Azadirachta indica*, *Urtica dioica* and dried sap of *Aloe secundiflora* leaves administered orally, decrease blood glucose levels in normal rats during an oral glucose tolerant test. This shows that the rats were sensitive to hypoglycaemic agents.
3. A single intraperitoneal injection of STZ (50 mg/kg) in normal fasted Wistar rats produces a reproducible and consistent model of diabetes in the laboratory conditions used. The rats show an increase in plasma glucose levels, food and water intake, and decrease in body weight compared to the control groups.
4. Treatment of STZ-induced diabetic rats with (80 mg/kg b.w.) *Azadirachta indica*, *Urtica dioica* and dried sap of *Aloe secundiflora* leaves significantly inhibits hyperglycaemic action of STZ.
5. In isolated intestinal segments of rabbit, 40 mg leaf extracts of *Azadirachta indica*, *Urtica dioica* and dried sap of *Aloe secundiflora* inhibits glucose absorption through the intestine.

5.3 Recommendations

1. Ethanol extracts of *Azadirachta indica*, *Urtica dioica* and dried sap of *Aloe secundiflora* leaves indicated they are potential antihyperglycaemics and can be used to manage type 2 diabetes. The modified syrup form improves their palatability, shelf life and makes them ready to use. Streamlining factors that influence the chemical composition of the

hypoglycaemic plants could greatly improve their efficacy in reduction of blood glucose levels.

2. Excessive use of ethanol extracts of *Azadirachta indica*, *Urtica dioica* and dried sap of *Aloe secundiflora* leaves should be avoided. Oral administration of the extracts beyond 80 mg/kg showed no additional positive effects. Alongside their hypoglycaemic activity, high concentrations showed controversial side effects. Thus a need to modulate the use of these extracts in the population. These extracts from the plants should be convenient, safe and effective in modulating blood glucose levels.

3. Future research may need to define targeted diabetic populations as pertains to disease classification and severity. It will also be important to further elucidate mechanisms of action of the hypoglycaemic plants, so that applicability to type 1 or type II diabetes can be clarified.

4. That research on ethanol extracts of *Azadirachta indica* and *Urtica dioica* leaves should be intensified and the hypoglycaemic components in these plants isolated and tested.

5. That research on dose-response effect of water extracts of *Azadirachta indica*, *Urtica dioica* and dried sap of *Aloe secundiflora* leaf on diabetic animals should be intensified in order to show their effect in type 1 or type II diabetic model.

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APPENDIX I

ALTITUDE, RAINFALL TEMPERATURE AND HUMIDITY DATA OF THE AREAS WHERE *ALOE SECUNDIFLORA*, *URTICA DIOICA* AND *AZADIRACHTA INDICA* LEAVES USED IN THE STUDY WERE COLLECTED.

Parameter	<u>Collection area.</u>		
	Samburu	Molo	Mombasa
Altitude (M)	1000- 1500	1500-2000	0
Mean annual rainfall (mm)	401-600	801-1200	801-1200
Mean annual minimum temperature (° C)	10-15	5-10	20-30
Mean annual maximum temperature (°C)	25-30	20-25	25-30
Relative humidity (%)	40-60	40-60	70

Source: National Atlas of Kenya, 2003.

APPENDIX II**PROTOCOL FOR DETERMINATION OF GLUCOSE**

Addition sequence	Blank ml	Standard ml	Test ml
Glucose reagent	1.0	1.0	1.0
Distilled water	.05		
Glucose standard		.05	
Sample			.05

APPENDIX III**FORMULAR FOR CONVERSION OF MMOL TO MG USED IN GLUCOSE DETERMINATION PROCEDURE**

$$1\text{mMol} = 18.0180 \text{ mg/Dl}$$

For calculation of glucose concentration, % C,

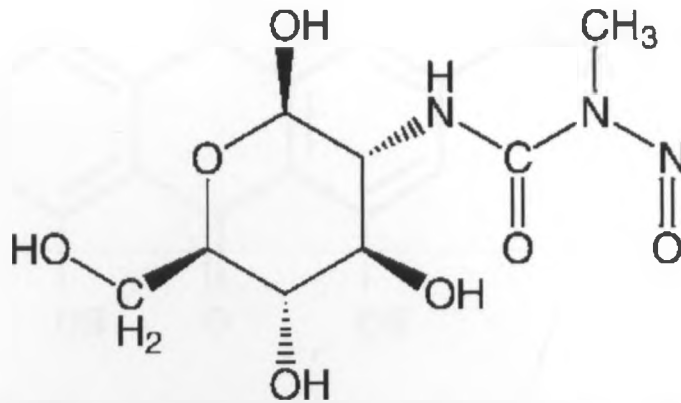
$$\%C = 100 \times D_s (\text{sample}) / D_s (\text{standard}) = \text{m/dl}$$

where;

D is the optical density.

APPENDIX IV

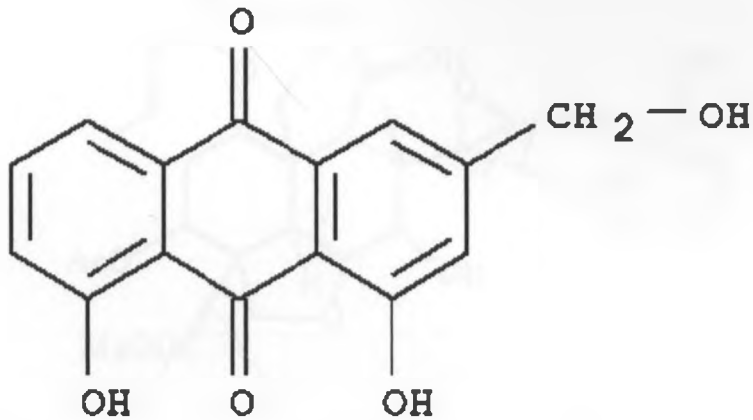
STRUCTURE OF STREPTOZOTOCIN



Chemical structure of Streptozotocin (STZ) a substrate with selective toxicity against pancreatic beta cells used to treat insulin producing beta-cell tumours (insulinoma) and to induce diabetes mellitus in experimental animals (Schein and Loftus, 1968).

APPENDIX V

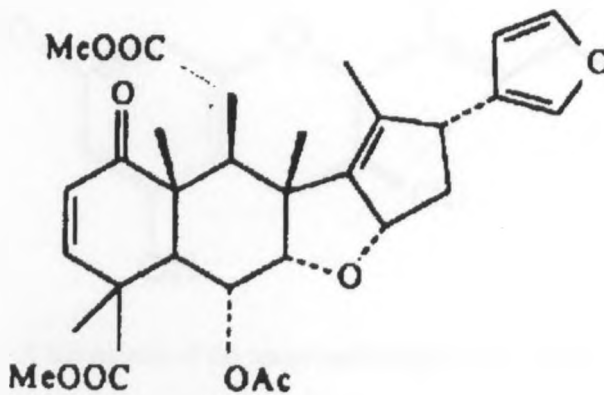
STRUCTURE OF ALOE EMODIN



One of the polysaccharide isolated from *Aloe vera* mucilaginous gel by the alcohol precipitation method (Gowda *et. al.*, 1991). Morgan showed that the mannose phosphate will bind to the insulin like growth factor receptor (Morgan, 1987).

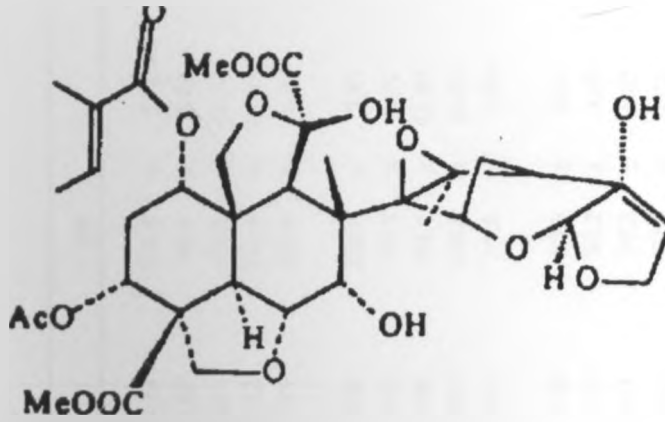
APPENDIX VI

STRUCTURE OF NIMBIN



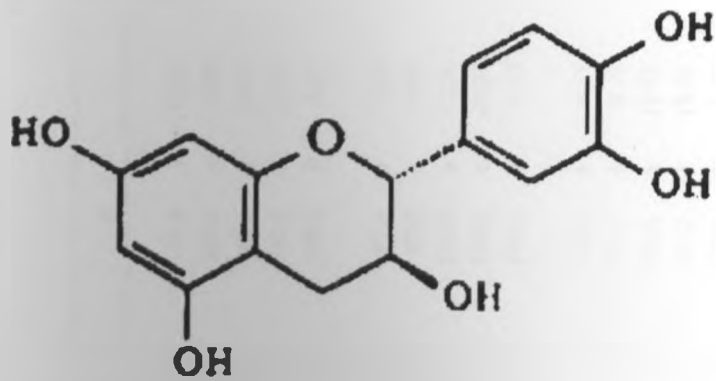
Isolated from Nimbidin. Nimbidin is a major crude bitter principle extracted from the oil of seed kernels of *A. indica* (Chopra *et. al.*, 1956).

STRUCTURE OF AZADIRACTIN



Isolated from Nimbidin, (a major crude bitter principle extracted from the oil of seed kernels of *A. indica* (Thakur *et. al.*, 1981)

STRUCTURE OF CATECHIN



A constituent of the neem leaf (Singh *et. al.*, 1996)

APPENDIX VII

Chronic effect of graded doses of 60% ethanol extract of *U. dioica*, *A. indica* and *A. secundiflora* on fasting blood glucose levels of normoglycaemic rabbits after 18 hour fasting.

Extract concentration		Blood glucose level mg/dL (mean \pm SD)											
		Duration in days											
		0	6	12	18	24	30						
10	A	96.6 \pm 10.60	97.4 \pm 12.42	98.8 \pm 8.73	100.2 \pm 7.79	101.6 \pm 5.94	104.4 \pm 7.09						
	B	100.0 \pm 6.44	100.4 \pm 4.98	99.4 \pm 4.98	100.4 \pm 4.62	99.6 \pm 4.83	100.2 \pm 5.26						
	C	113.6 \pm 16.55	104.6 \pm 13.15	107.4 \pm 18.12	95.0 \pm 10.37	103.6 \pm 15.50	94.2 \pm 9.12						
	D	112.4 \pm 16.57	110.2 \pm 17.11	111.0 \pm 17.39	109.0 \pm 18.32	111.4 \pm 16.89	109.2 \pm 17.58						
	E	112.0 \pm 10.58	109.2 \pm 8.53	105.4 \pm 8.44	103.2 \pm 7.29	103.0 \pm 6.60	101.0 \pm 6.32						
20	A	96.0 \pm 7.18	97.4 \pm 8.79	100.8 \pm 8.93	103.0 \pm 6.28	100.4 \pm 7.89	99.4 \pm 6.43						
	B	98.6 \pm 6.15	97.8 \pm 6.91	98.6 \pm 6.07	98.2 \pm 6.22	98.8 \pm 6.91	99.4 \pm 5.94						
	C	108.8 \pm 19.25	101.8 \pm 11.56	105.6 \pm 19.14	94.4 \pm 9.68	104.4 \pm 16.89	92.0 \pm 9.62						
	D	111.2 \pm 15.35	108.0 \pm 15.07	110.0 \pm 16.16	106.8 \pm 15.80	108.8 \pm 16.54	105.6 \pm 16.47						
	E	109.0 \pm 8.40	106.8 \pm 9.65	102.4 \pm 6.88	101.6 \pm 8.35	99.8 \pm 5.26	98.8 \pm 6.69						
40	A	95.4 \pm 8.08	93.6 \pm 6.65	101.4 \pm 6.66	102.4 \pm 8.38	98.6 \pm 8.62	101.6 \pm 6.88						
	B	96.6 \pm 6.07	97.4 \pm 4.98	96.0 \pm 4.58	97.0 \pm 4.30	96.2 \pm 4.49	96.8 \pm 4.92						
	C	106.2 \pm 19.88	100.6 \pm 12.78	100.4 \pm 16.98	95.2 \pm 10.85	97.2 \pm 16.05	91.8 \pm 9.96						
	D	103.0 \pm 14.78	101.2 \pm 12.46	99.4 \pm 14.22	98.0 \pm 12.63	99.2 \pm 14.82	97.6 \pm 12.86						
	E	104.4 \pm 7.99	102.8 \pm 8.23	98.0 \pm 6.40	97.0 \pm 5.70	96.0 \pm 5.96	96.6 \pm 4.39						

APPENDIX VII contd.

Chronic effect of graded doses of 60% ethanol extract of *U. dioica*, *A. indica* and *A. secundiflora* on fasting blood glucose levels of normoglycaemic rabbits after 18 hour fasting.

Extract concentration	Blood glucose level mg/dL (mean \pm SD)												
	Duration in days												
	0		6		12		18		24		30		
80	A	93.6	\pm 8.05	94.6	\pm 6.95	100.0	\pm 7.11	98.2	\pm 9.25	97.0	\pm 6.78	97.0	\pm 9.54
	B	96.8	\pm 6.91	96.0	\pm 8.31	96.8	\pm 7.26	96.4	\pm 6.95	96.2	\pm 7.29	97.6	\pm 6.84
	C	105.0	\pm 19.71	101.4	\pm 12.76	95.0	\pm 14.88	92.0	\pm 16.48	89.8	\pm 14.11	88.2	\pm 10.87
	D	99.2	\pm 12.56	99.8	\pm 10.59	92.0	\pm 10.12	92.8	\pm 8.26	91.2	\pm 11.12	91.8	\pm 9.07
	E	104.6	\pm 7.92	99.6	\pm 4.67	97.6	\pm 6.23	94.4	\pm 6.02	94.4	\pm 6.02	92.6	\pm 5.22
100	A	98.2	\pm 10.03	97.8	\pm 10.11	101.6	\pm 9.24	101.0	\pm 8.60	98.8	\pm 7.05	98.4	\pm 5.87
	B	93.20	\pm 8.17	94.0	\pm 7.14	92.6	\pm 6.69	93.6	\pm 6.80	92.8	\pm 6.53	93.8	\pm 6.83
	C	101.6	\pm 17.02	97.2	\pm 16.53	91.0	\pm 14.30	87.2	\pm 12.21	82.4	\pm 10.38	79.2	\pm 10.40
	D	96.4	\pm 9.81	92.6	\pm 10.74	89.6	\pm 9.10	86.0	\pm 10.77	88.4	\pm 7.70	85.2	\pm 8.29
	E	104.6	\pm 7.92	99.6	\pm 4.67	97.6	\pm 6.23	94.4	\pm 6.02	94.4	\pm 6.02	92.6	\pm 5.22

APPENDIX VIII

Acute effect of effective dose of 60% ethanol extract of *U. dioica*, *A. indica* and *A. secundiflora* on fasting blood glucose levels of diabetic rats after 18 hour fasting.

Groups	Blood glucose level mg/dL (mean \pm SD)														
	Duration in hrs														
	0		1		2		3		4						
1	97.3	\pm 8.45	***	101.5	\pm 8.84	***	95.3	\pm 7.83	***	96.1	\pm 8.44	***	94.4	\pm 8.18	***
2	454.5	\pm 67.40		464.1	\pm 68.03		455.5	\pm 65.84		451.6	\pm 64.28		444.3	\pm 62.80	
3	259.4	\pm 130.47	***	273.1	\pm 141.07	***	232.2	\pm 134.04	***	178.3	\pm 104.49	***	138.0	\pm 95.23	***
4	320.3	\pm 76.36	***	307.9	\pm 81.98	***	275.1	\pm 85.21	***	234.4	\pm 79.61	***	193.4	\pm 82.82	***
5	301.1	\pm 70.28	***	290.1	\pm 77.33	***	279.0	\pm 69.47	***	281.7	\pm 81.89	***	217.2	\pm 62.75	***
6	269.3	\pm 54.57	***	287.5	\pm 58.89	***	223.4	\pm 46.47	***	199.4	\pm 41.94	***	164.6	\pm 46.25	***

Comparisons were made between: (*) Group 2 and group 1, 3, 4, 5, 6 ; The symbol represent statistical significance. * P < 0.05, ** P < 0.01 and *** P < 0.001.

Three groups consisting of diabetic rats were administered with 80 mg/kg of plant extracts *A. secundiflora* (group 3), *A. indica* (group 4), *U. dioica* (group 5) and the fourth and the fifth groups consisting of diabetic rats were given placebo as control (group 2), and glibenclamide (positive control, group 6) respectively. Group 1 consisted of non-diabetic normal rats. Change in the blood glucose levels were studied at 0, 1,2,3,4 hours.

APPENDIX IX

Chronic effect of effective dose of 60% ethanol extract of *U. dioica*, *A. indica* and *A. secundiflora* on fasting blood glucose levels of diabetic rats after 18 hour fasting.

Duration in days	Blood glucose level mg/dL (mean \pm SD)											
	Group treatments											
	1		2		3		4		5		6	
-12	91	\pm 5.96	88	\pm 7.16	91	\pm 5.06	87	\pm 3.77	88	\pm 2.44	89	\pm 2.69
-8	91	\pm 6.06	88	\pm 6.20	90	\pm 5.04	88	\pm 3.87	88	\pm 3.77	91	\pm 3.51
-4	94	\pm 9.09 ***	354	\pm 59.89	449	\pm 33.89 **	398	\pm 44.52	363	\pm 43.57	388	\pm 24.16
0	96	\pm 10.08 ***	381	\pm 72.44	435	\pm 47.88	398	\pm 49.36	356	\pm 41.16	378	\pm 18.56
4	104	\pm 9.44 ***	409	\pm 80.27	395	\pm 42.32	362	\pm 59.9	349	\pm 32.55	326	\pm 23.21 *
8	101	\pm 8.07 ***	439	\pm 82.58	382	\pm 43.30	373	\pm 50.74	341	\pm 61 *	314	\pm 16.96 **
12	94	\pm 5.38 ***	438	\pm 57.60	336	\pm 19.8 ***	362	\pm 42.77 *	329	\pm 65.17 *	276	\pm 17.06 ***
16	94	\pm 7.78 ***	456	\pm 60.00	331	\pm 36.54 ***	342	\pm 38.48 **	302	\pm 77.74 **	271	\pm 17.82 ***
20	95	\pm 8.85 ***	462	\pm 47.07	227	\pm 25.79 ***	331	\pm 34.00 ***	293	\pm 74.17 ***	264	\pm 16.37 ***
24	93	\pm 6.85 ***	486	\pm 48.38	214	\pm 24.44 ***	311	\pm 30.95 ***	282	\pm 68.51 ***	254	\pm 17.52 ***
28	98	\pm 8.98 ***	483	\pm 42.78	171	\pm 19.55 ***	290	\pm 29.18 ***	274	\pm 72.49 ***	245	\pm 15.23 ***
32	98	\pm 9.43 ***	491	\pm 43.76	128	\pm 19.24 ***	267	\pm 33.41 ***	268	\pm 74.06 ***	227	\pm 20.84 ***
36	100	\pm 6.90 ***	495	\pm 37.83	75	\pm 12.18 ***	245	\pm 37.97 ***	254	\pm 71.43 ***	213	\pm 13.25 ***
40	99	\pm 8.71 ***	508	\pm 44.20	62	\pm 6.40 ***	152	\pm 18.09 ***	241	\pm 64.80 ***	194	\pm 34.75 ***

*P<0.05, ** P<0.01, *** P<0.001 when compared with diabetic control

Four groups consisting of diabetic rats were administered with 80 mg/kg of plant extracts *A. secundiflora* (Group 3), *A. indica* (Group 4), *U. dioica* (Group 5) and placebo as control (Group 2). The fifth set consisting of diabetic rats was administered glibenclamide (positive control, Group 6). Group 1 consisted of non-diabetic normal rats. Change in the blood glucose levels was studied after every four days. n=7 in all groups.